# The rotator cuff: from bench to bedside

## Developments in tissue engineering, surgical techniques and pathogenetic factors


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# The rotator cuff: from bench to bedside

## Developments in tissue engineering, surgical techniques and pathogenetic factors

# De rotator cuff: van bench to bedside

## Ontwikkelingen in de tissue engineering, chirurgische technieken en pathogenetische factoren

(met een samenvatting in het Nederlands)

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## TABLE OF CONTENTS

## PUBLICATIONS CONTRIBUTING TO THIS THESIS

1. **Chapter 2: U.G. LONGO**, A. Lamberti, N. Maffulli, and V. Denaro. *Tissue engineered biological augmentation for tendon healing* **British Medical Bullettin** 2011;98:31-59
2. **Chapter 3: U.G. LONGO**, A. Lamberti, N. Maffulli, and V. Denaro. *Tendon augmentation grafts* **British Medical Bullettin** 2010;94:165-88
3. **Chapter 4:** N. Maffulli, **U.G. LONGO**, and V. Denaro. *Novel approaches for the management of tendinopathy* **Journal of Bone and Joint Surgery – American Volume** 2010;92(15):2604-13
4. **Chapter 5: U.G. LONGO**, F. Franceschi, L. Ruzzini, C. Rabitti S. Morini, N. Maffulli, and V. Denaro. *Histopathology of the supraspinatus tendon in rotator cuff tears.* **American Journal of Sports Medicine** 2008;36(3):533-8.
5. **Chapter 6: U.G. LONGO,** F. Franceschi, L. Ruzzini, C. Rabbiti, S. Morini, N. Maffulli, F. Forriol, and V. Denaro. *Light microscopic histology of supraspinatus tendon ruptures.* **Knee Surgery, Sports Traumatology, Arthroscopy** 2007;15(11):1390-4.
6. **Chapter 7: U.G. LONGO**, F. Franceschi, L. Ruzzini, F. Spiezia, N. Maffulli, and V. Denaro. *Higher fasting plasma glucose levels within the normoglycemic range and rotator cuff tears* **British Journal of Sports Medicine**. 2009;43:284-287.
7. **Chapter 8: U.G. LONGO,** F. Franceschi, F. Spiezia, F. Forriol, N. Maffulli, and V. Denaro. *Triglycerides and total serum cholesterol in rotator cuff tears: do they matter?* **British Journal of Sports Medicine** 2010 Oct;44(13):948-51.
8. **Chapter 9:** F. Franceschi, L. Ruzzini, **U.G. LONGO**, F.M. Martina, B.B. Zobel, N. Maffulli, and V. Denaro. *Equivalent Clinical Results of Arthroscopic Single-Row and Double-Row Suture Anchor Repair for Rotator Cuff Tears: A Randomized Controlled Trial* **American Journal of Sports Medicine**. 2007;35(8):1254-60.
9. **Chapter 10:** R. Castricini **U.G. LONGO** R, M. De Benedetto, N. Panfoli, P. Pirani, R Zini, N. Maffulli, and V. Denaro. *Platelet-rich plasma augmentation for arthroscopic rotator cuff repair: a randomised controlled trial* **American Journal of Sports Medicine** 2011;39(2):258-65
10. **Chapter 11: U.G. LONGO**, D. Saris, R.W. Poolman, A. Berton, and V. Denaro. *Instruments to assess patients with rotator cuff pathology: a systematic review of measurement properties* **Knee Surgery, Sports Traumatology, Arthroscopy** 2011 Dec 20. [Epub ahead of print]
11. **Chapter 12: U.G. LONGO**, F. Forriol, S. Campi, N. Maffulli, and V. Denaro. *Animal models for translational research on shoulder pathologies: from bench to bedside* **Sports Medicine and Arthroscopy Review** 2011;19(3):184-93

# Chapter 1

## Introduction and aims

This thesis originates from the difficulties that arise from management of patients with rotator cuff tears.

Rotator cuff tendon tears account for more than 4.5 million physician visits per year, and over 250,000 RC repair surgeries performed annually in the United States [359]. Despite the relevance of the topic, causes of rotator cuff disease are still debated [358]. The histopathological features of the ruptured rotator cuff tendon have not been previously well established [357]. Furthermore, the systemic factors that may cause rotator cuff tears are still unknown [394].

Results of surgery for rotator cuff tendon tears have been also contradictory. Given the limited ability for healing of the rotator cuff, several strategies have been proposed to enhance tendon healing. Rotator cuff tendon healing could be theoretically improved by 2 approaches:

1. Trying to improve the **mechanics** of the surgical repair (*i.e.*, modifying the common surgical techniques towards more complex double row suture anchors configuration of the repair). Restoring the anatomic footprint may theoretically improve the healing and mechanical strength of repaired tendons [362]. A single row of suture anchors may not be effective for this purpose. A double row of suture anchors increases the tendon-bone contact area, reconstituting a more anatomic configuration of the rotator cuff footprint.
2. Trying to improve **biology** of the repaired tendon (i.e., using factors and cytokines, gene therapy, tendon augmentation graft and tissue engineering with mesenchymal stem cells) [369, 372]. To date, there are no data from randomised trials assessing the efficacy and safety of platelet rich plasma augmentation for rotator cuff repair.

Orthopaedic surgeons have classically embraced innovations or new techniques on the basis of limited research evidence. With the increase of health care costs due to new techniques it may be necessary to move towards using interventions based on sound evidence established through high quality clinical research with patient-relevant outcomes and demonstrated cost-effectiveness.

There is need to evaluate in randomised controlled trials whether new double row techniques and platelet rich plasma augmentation will improve rotator cuff healing.

The emerging field of tissue engineering holds the promise to use new techniques for tendon augmentation and repair. Preliminary studies support the idea that these techniques can provide an alternative for tendon augmentation with great therapeutic potential. One of the main limitation of the application of new techniques is the lack of a good animal model for experimental rotator cuff tear.

The aims of this thesis are threefold to study the pathogenesis of rotator cuff tears, to evaluate the safety and efficacy of new proposed methods to improve healing of the repaired rotator cuff tendon, and highlight limitations for future research and growing points in the field. Detailed aims of this thesis are:

- To study the pathogenesis of rotator cuff tears
  - to evaluate the histopathological features of rotator cuff tendon
  - to evaluate the influence of systemic triglycerides and glucose level on the development of rotator cuff tendon tears
- To evaluate efficacy and safety of new strategies to improve rotator cuff healing in randomised controlled trials
  - Mechanics: To compare single versus double row suture anchors techniques in a randomised controlled trial
  - Biology: To compare the efficacy and safety of augmentation with platelet rich plasma (PRP) for arthroscopic rotator cuff repair compared with non-augmented repair of the rotator cuff in a randomised controlled trial
- To review the state of the art on tendon healing, scores for evaluating patients with rotator cuff tears and animal models, highlighting future areas of interest and practical limitations.

## SPECIFIC AIMS OF THIS THESIS

### 1. Tissue engineered biological augmentation for tendon healing

Tendon tears are generally managed by direct suturing techniques [360]. However, tendon healing rate is relatively slow compared with other connective tissues, in particular because of its poor vascularization. The most common form of tendon healing is by scar formation. This affects function, and it is accompanied by an increased risk of further damage.

Because of the limited capacity for self-healing, management of tendon injuries is complex, and several issues have to be addressed, including prolonged management time, possible weakness in the affected area, recurrent injury, and loss of function. Surgical management allows early rehabilitation and reduces the rate of re-rupture, but has various complications, such as infection, nerve damage, and scarring, which may compromise the outcome. For these reasons, new approaches are required to improve tendon healing.

In the last few decades, several emerging strategies - including growth factors and cytokines, gene therapy and tissue engineering with mesenchymal stem cells (MSC) - have been proposed to enhance tendon healing. They hold the promise to yield more successful outcomes for the management of patients with tendon pathology.

Therefore the aim of the review presented in **Chapter 2** is to review the current knowledge in the field of tissue engineered biological augmentation for tendon healing [371].

*<u>Question addressed:</u> Can growth factors, cytokines, gene therapy and tissue engineering enhance tendon healing?*

## 2. Tendon augmentation grafts

Management of large and massive rotator cuff tears tendon can present a dilemma to the orthopaedic surgeon. Tendon augmentation can provide a more effective management option producing a stronger construct [197, 396]. Surgeons may tackle these injuries using autografts, allografts, xenografts and tendon prosthesis [40]. Allografts and xenografts have become increasingly popular for tendon and ligament repair to overcome the limited availability and donor site complication encountered with the use of autograft tissue [114]. Several new tissue engineered materials have been introduced: artificial polymers, biodegradable films and biomaterials derived from animals or human, using a combination of principles of engineering and biology [40]. As limitations of previous generations of biologically derived materials are overcome, many new and impressive applications for biomaterials are being examined.

Therefore the aim of the reviews presented in **Chapter 3** is to review the current state of knowledge in the field of biomaterials for augmentation of rotator cuff and Achilles tendon injuries [370].

*Question addressed: Can biomaterials provide a reliable tool in patients with tendon tears?*

## 3. Novel approaches for the management of tendinopathy

Most major tendons, such as the rotator cuff, are vulnerable to overuse, which induces pathological changes in the tendon [530]. Despite an abundance of therapeutic options, very few randomised prospective, placebo controlled trials have been conducted to assist in choosing the best evidence-based management. Tendinopathy is a failed healing response of the tendon, and tendon pathology is consistent across sides. Even an experienced pathologist is not able to recognize where a tendon sample is harvested from [401].

Managements that have been investigated using a randomised controlled trial design include nonsteroidal anti-inflammatory medications [37, 39, 281], eccentric exercise [151, 200, 510, 541, 546], glyceryl trinitrate patches [295, 489, 490], sclerosing injections [262], platelet rich plasma, ultrasound [118], and shock wave treatment [11, 121, 135, 222, 332, 513, 545, 567, 571, 604, 605, 610].

Therefore the aim of the review presented in **Chapter 4** is to review the best available evidence for the management of tendinopathy and provide a comprehensive and up-to-date review of the development of future modalities for treatment [395].

*Question addressed: What is the best available evidence for the management of tendinopathy?*

### 4. Histopathology of the supraspinatus tendon in rotator cuff tears.

Systemic histopathological studies examining pathological findings and their distribution in rotator cuff tendons are lacking in literature.

To date, the histopathological features of the macroscopic intact portion of the rotator cuff tendon in patients with a rotator cuff tear have not been studied [357].

Therefore, the aim of the study presented in **Chapter 5** is to analyse the histopathological features of the macroscopic intact portion of surgical specimens of supraspinatus tendon from patients with rotator cuff tears [364].

*Question addressed: Does macroscopically intact supraspinatus tendon show changes that may be shown by microscopic examination, and can represent the pathogenic precursor to a subsequent rotator cuff tear?*

### 5. Light microscopic histology of supraspinatus tendon ruptures

Many studies have attempted to correlate the incidence of rotator cuff tears with the compression of the tendons by direct pressure from surrounding soft tissue or bony impingement. In 1972, Neer [459] proposed that the majority of rotator cuff tears result from mechanical compression of the tendons under the coracoacromial arch. Successively, Bigliani [69] reported a correlation between acromial morphology and rotator cuff tears, showing that the Type III acromion was present in the majority of rotator cuff tears. Many authors [202, 203] showed that pathologic changes can occur at the bursal side of the rotator cuff, suggesting a role of friction and rubbing played from the undersurface of the acromion. On the other hand, various authors advocated that intrinsic factors instituted rotator cuff pathology [555, 556]. Specifically, the proposed causes of intrinsic degeneration are aging, tensile overload and microvascular supply.

To date, there is no consensus on the localization of histopathological changes in rotator cuff tears.

Therefore, the aim of the study presented in **Chapter 6** is to compare the histopathological features of the gleno-humeral and subacromial portions of the rotator cuff [365].

*Question addressed: Does it exist any differences in the localization of histopathological changes in rotator cuff tears?*

### 6. Higher fasting plasma glucose levels within the normoglycemic range and rotator cuff tears

There is a possible relationship between hyperglycaemia and collagen structure alterations [526, 527]. At tissue level, tendons may be directly affected by non-enzymatic glycosylation processes which change collagen cross-links [48]. One of the underlying mechanisms

of this cross-linking is the formation of advanced glycation endproducts [431]. The normal fasting plasma glucose level has been defined as less than 100 mg per decilitre (5.55 mmol per litre) [638]. Whether higher fasting plasma glucose levels within this range independently predict rotator cuff tear is unknown.

To our knowledge, no studies have focused on the correlation between plasma glucose levels and rotator cuff tears.

Therefore, the aim of the study presented in **Chapter 7** is to undertake a frequency-matched case-control study of the plasma glucose level obtained from non-diabetic patients undergoing arthroscopic rotator cuff repair, and compared with a matched control group of patients of a similar age [366].

*Question addressed: Is there a difference in plasma glucose level in patients presenting with an arthroscopically confirmed lesion of the rotator cuff and a control group?*

### 7. Triglycerides and total serum cholesterol in rotator cuff tears: do they matter?

There are data on the possible relationship between high serum lipid concentration and complete rupture of the Achilles tendon [417, 485]. However, to our knowledge, no studies have focused on the correlation between serum lipid levels and rotator cuff tears.

Therefore, the aim of the study presented in **Chapter 8** is to perform a cross-sectional study of the serum triglyceride concentration and total serum cholesterol concentration in patients undergoing arthroscopic rotator cuff repair, and compared them with a control group of patients of a similar age [367].

*Question addressed: Is there a difference in serum lipid concentration in patients presenting with an arthroscopically confirmed lesion of the rotator cuff and a control group?*

### 8. Equivalent Clinical Results of Arthroscopic Single-Row and Double-Row Suture Anchor Repair for Rotator Cuff Tears: A Randomized Controlled Trial

Arthroscopic management of rotator cuff tears has evolved from simple debridement to arthroscopic repair providing anatomic reconstruction [32].

Restoring the anatomic footprint may improve the healing and mechanical strength of repaired tendons [32]. A single row of suture anchors may not be effective for this purpose. A double row of suture anchors increases the tendon-bone contact area, reconstituting a more anatomic configuration of the rotator cuff footprint [32].

Therefore the aim of the randomised controlled trial presented in **Chapter 9** is to compare the clinical and structural outcome of single versus double row suture anchor repair of a rotator cuff tear [198].

*Question addressed: Is there any difference in clinical and imaging outcome between single row and double row suture anchor technique repairs of rotator cuff tears?*

### 9. Platelet-rich plasma augmentation for arthroscopic rotator cuff repair: a randomised controlled trial

The rotator cuff has limited ability to heal back to its insertion on the humerus following repair, possibly because of the poor vascularization of tendon tissue, and also because the histopathological changes which accompany a rupture are localized not only at the site of rupture but also in the macroscopic intact tendon portion, suggesting more generalised involvement of the tendon. Given this limited ability for healing, several strategies - including growth factors and cytokines, gene therapy, tendon augmentation graft and tissue engineering with mesenchymal stem cells - have been proposed to enhance tendon healing. Several growth factors are upregulated during RC healing, and they may be used to augment RC repairs.

Platelet-rich plasma (PRP) and platelet-rich fibrin matrix (PRFM), or autologous platelet-derived growth factors, are bioactive components of whole blood, which are now being widely tested in different fields of medicine to aid healing in tissue with poor healing potential [189, 193, 244, 322, 442, 443, 552-554]. Cascade® Autologous Platelet System (MTF, Musculoskeletal Trasplant Foundation) is a completely autologous platelet biologic matrix [414], with a high concentration of viable platelets, extracted from a small amount of the patient's own blood, spun through a centrifugation process and resulting in a dense suturable PRFM that can be delivered directly to the tear site and sutured in place to potentially stimulate a reparative healing response for soft tissue and bone repair.

To date, there are no data from randomised trials assessing the efficacy and safety of PRFM for augmentation of RC repair.

Therefore the aim of the study presented in **Chapter 10** is evaluate the efficacy and safety of PRFM for augmentation of RC repair [105].

*Question addressed: Does PRFM improve the clinical and structural outcome of patients undergoing rotator cuff repair?*

### 10. Instruments to assess patients with rotator cuff pathology: a systematic review of measurement properties

Different measurement instruments have been developed for shoulder pain. Outcomes measures typically fall into two broad categories: general health, joint- and disease- specific. Generic measures are designed to assess functional status regardless of an individual's disease/disorder [363, 373]. Condition-specific measures are designed to be sensitive to the specific disease/disorder of interest. Even though several outcome measures are available

for studying patients with impairment of shoulder function [375], often there is no consensus on which instrument is most suitable for what purpose, as only a few of these instruments have been validated in patients with rotator cuff pathology. Moreover, not all instruments are developed with use of strict quality criteria [515]. Recently, these standardised criteria were published by the COSMIN group. COSMIN stands for Consensus-based Standards for the selection of health Measurement Instruments [445,446]. According to the COSMIN guidelines, the quality of a measurement instrument is described by three quality domains: reliability, validity and responsiveness. Reliability contains the measurement properties internal consistency, reliability and measurement error, whereas validity contains content validity, construct validity and criterion validity [574].

To date, there are no systematic review that use the COSMIN guidelines to analyse the methodological quality of studies on the measurement properties of rotator cuff questionnaires.

Therefore the aim of the study presented in **Chapter 11** is to obtain an overview of the methodological quality of studies on the measurement properties of rotator cuff questionnaires and to describe how well various aspects of the design and statistical analyses of studies on measurement properties are performed using the COSMIN guidelines [374].

*Question addressed: Which is the best rotator cuff questionnaire for patients with rotator cuff disease?*

## 11. Animal models for translational research on shoulder pathologies: from bench to bedside

The use of animal models to study human pathology is valuable in many fields. Several animal models have been used for *in vivo* and *in vitro* shoulder research. *In vitro* models, consisting of cadaveric specimens are useful in providing basic understanding of the functioning of the shoulder [62,63]. *In vivo* models provide the means to model phenomena, such as tendon healing process, tendon degeneration, instability and adaptive responses to surgery. Basically, human specimens are more suitable for these models than are animal specimens whenever anatomy, size and kinematics are important. However, there are some disadvantages in using the human model. One problem is the difficulty in obtaining fresh human specimens, especially from younger subjects. These disadvantages of human specimens force a search for alternative animal models [161].

The presence of a validated animal model would enable in-depth studies on the aetiology, molecular mechanisms and potential treatments of different shoulder pathologies, as animals are more homogeneous and are easier to control than humans [381]. However, given the differences among different species, it is sometimes difficult to reproduce reliable diseases phenotype in animals. While each of these animal species may possess bony and soft-tissue anatomy with varying similarities to the human shoulder, none is the same [161].

Each of them has peculiar advantages or disadvantages. An ideal animal shoulder model should have similar anatomy and function as human, an intrasynovial injury environment, possibility to develop a chronic injury condition, tendon size similar to human (to allow for standard techniques of repair), muscle atrophy, stiffening, and fatty infiltration after a tendon tear, absence of spontaneous tendon healing or scar formation without treatment, incidence of tendon re-tear, the ability to control postoperative mechanical loading on the repair [161]. There are obvious stark marked anatomical differences between quadripeds and bipeds, especially in the forelimbs. Most of the animals used in experimental settings are quadrupeds, using the forelimbs for weight-bearing during locomotion, with no or minimal overhead activity. Also, differences exist between absolute quadrupeds (e.g. goat, sheep, calf) and quadrupeds also working with their hands standing on their legs (e.g. rat, squirrels, monkey). Quadrupeds use its supraspinatus to accelerate a pendulum, while in humans it raises their arm and acts at a disadvantage against gravity and under great strain [598]. While movement of quadrupedal shoulders is largely restricted to the sagittal plane, those of bipedal primates can additionally rotate and move in the coronal plane, thereby allowing much more mobility [598]. From an evolutionary point of view, this necessitates adaptations in the architecture of the bone and soft tissue [598].

The absence of validated animal models for the study of shoulder pathology challenges the research on this field.

Therefore, the aim of the review presented in **Chapter 12** is to evaluate the role of different animal models in shoulder research [361].

*Question addressed: What is the best animal model to be used in rotator cuff research?*

# Strategies to improve tendon healing

# Chapter 2

## Tissue engineered biological augmentation for tendon healing. A systematic review

## ABSTRACT

**Introduction:** Tendon injuries give rise to significant morbidity. In the last few decades, several techniques have been increasingly used to optimize tendon healing.

**Sources of data:** We performed a comprehensive search of PubMed, Medline, Cochrane, CINAHL, and Embase databases using various combinations of the commercial names of each scaffold and the keywords "tendon", "rotator cuff", "supraspinatus tendon", "Achilles tendon", "growth factors", "cytokines", "gene therapy", "tissue engineering", "mesenchymal", and "stem cells" over the years 1966–2009. All articles relevant to the subject were retrieved, and their bibliographies were hand searched for further references in the context to tissue engineered biological augmentation for tendon healing.

**Areas of agreement:** Several new techniques are available for tissue engineered biological augmentation for tendon healing, growth factors, gene therapy and mesenchimal stem cells.

**Areas of controversy:** Data are lacking to allow definitive conclusions on the use of these techniques for routine management of tendon ailments.

**Growing points:** The emerging field of tissue engineering holds the promise to use new techniques for tendon augmentation and repair. Preliminary studies support the idea that these techniques can provide an alternative for tendon augmentation with great therapeutic potential.

**Areas timely for developing research:** The optimization strategies discussed in this article are currently at an early stage of development. Whilst these emerging technologies may develop into substantial clinical treatment options, their full impact needs to be critically evaluated in a scientific fashion.

## INTRODUCTION

Tendon disorders are frequent, and are responsible for much morbidity both in sport and the workplace. Tendon pathology can broadly be divided into tendon tears or tendinopathy [21]. Tendon tears are generally managed by direct suturing techniques. However, tendon healing rate is relatively slow compared with other connective tissues, in particular because of its poor vascularization. Another factor which may contribute to poor tendon healing is that the histopathological changes are not only localized at the site of rupture or tendinopathy, but also in the macroscopic intact tendon portion.

The most common form of tendon healing is by scar formation. This affects function, and it is accompanied by an increased risk of further damage. A further problem for tendon healing is the formation of adhesions which connect the tendon to surrounding tissues [260, 303]. The direct consequence of scarring and adhesions formation is impaired gliding motion, with loss of joint motion and function, and reduced quality of life [303]. Because of the limited capacity for self healing, management of tendon injuries is complex, and several issues have to be addressed, including prolonged management time, possible weakness in the affected area, recurrent injury, and loss of function [91, 529]. Surgical management allows early rehabilitation and reduces the rate of re-rupture, but has various complications, such as infection, nerve damage, and scarring, which may compromise the outcome. For these reasons, new approaches are required to improve tendon healing [153, 283, 335, 337, 344, 388, 397, 535, 579, 655].

In the last few decades, several emerging strategies - including growth factors and cytokines, gene therapy and tissue engineering with mesenchymal stem cells (MSC) - have been proposed to enhance tendon healing. They hold the promise to yield more successful outcomes for the management of patients with tendon pathology.

We review the current knowledge in the field of tissue engineered biological augmentation for tendon healing.

## METHODS

### Literature search and data extraction

We performed a comprehensive search of PubMed, Medline, Cochrane, CINAHL, and Embase databases using various combinations of the commercial names of each scaffold and the keywords “tendon”, “rotator cuff”, “supraspinatus tendon”, “Achilles tendon”, “growth factors”, “cytokines”, “gene therapy”, “tissue engineering”, “mesenchymal”, and “stem cells” over the years 1966–2010. All articles relevant to the subject were retrieved, and their bibliographies hand searched for further references in the context to tissue

engineered biological augmentation for tendon healing. Given the linguistic capabilities of the research team, we considered publications in English, Italian, French, Spanish and Portuguese. The search was limited to articles published in peer-reviewed journals. We excluded from our investigation case reports, literature reviews, and letter to editors. Eligible studies had to report on tissue engineered biological augmentation for tendon healing. Articles reporting on tissue engineered biological augmentation for ligament, muscle, cartilage, bone healing were excluded from the study.

## RESULTS

### *1. Growth factors and cytokines*

Growth factors are signalling molecules involved in cell chemotaxis, proliferation, matrix synthesis, and cell differentiation [240,241]. They also play an important role in regulation of the phases of tendon healing. After their release from platelets, polymorphonuclear leukocytes, and macrophages in the wound site, growth factors bind to cell surface receptors determining intracellular changes to DNA synthesis and expression, which result in induction of neovascularisation and chemotaxis, along with stimulation of fibroblast proliferation and collagen synthesis [581-585].

In animal models, growth factors are effective in increasing the cellularity and overall tissue volume at the repair site, resulting in increased failure loads on biomechanical testing. However, these failure loads become less significant when they are normalized to the volume or cross-sectional area of the repaired tissue [581-585]. This implies that growth factors are able to improve the strength of the repair by promoting the formation of more scar tissue (i.e., the structural properties are improved but the material properties are not improved). Excessive scar tissue at the healing attachment site may predispose patients to impingement post-operatively [303]. The ultimate outcome of the repair depends on both pullout strength and stiffness. Stiffness and creep may be more important parameters. Ideally, biologic therapies are able to induce tissue formation with material properties close to that of normal tissue.

Growth factors can be delivered to the site of injury by direct application. This is the most straight forward method, and can be achieved via local injection, or by using impregnated sutures or scaffolds. Using impregnated sutures or scaffolds has the advantage of delivering the growth factor to the specific area of injury. The disadvantage of overflow loss, associated with local injection, can be avoided with this technique. However, local injection is comparatively non-invasive, simple and quick. The main disadvantage of direct application is that growth factors only remain at the site for a short duration of time. As that tendon healing continues for months to years, this short duration of presence of

**Table 1:** Growth Factors

| Author | Tendon | Type of study | Model | Object of study |
|---|---|---|---|---|
| **Chen et al 2008** [113] | Flexor digitorum profundus | *In vivo* and *in vitro* | Chicken | CTGF, TGF-β, VEGF, IGF-I, bFGF, PDGF-B |
| **Anitua et al 2009** [29] | skin, synovium and tendon | *In vitro* | Human | PRGF |
| **Hou et al 2009** [266] | Achilles | *In vitro* and *in vivo* | Rabbit | TGF-β1 |
| **Klass et al 2009** [308] | Flexor tendons | *In vitro* | Rabbit | TGF- β1 |
| **Ricchetti et a 2008** [533] | Patellar | *In vivo* | Mouse | IL-10 |
| **Thomopoulos et al 2009** [632] | Flexor tendons | *In vivo* | Dog | PDGF-BB |
| **Yamada et al 2008** [683] | digital extensor tendons | *In vitro* | Bovine | rhOP-1 |
| **Abrahamsson et al 1991** [5] | Flexor Tendons | *In vitro* | Rabbit | rh-IGF-I, FCS |
| **Abrahamsson et al 1997** [3] | Flexor tendons | *In vitro* | Rabbit | h-IGF-II, h-IGF-I |
| **Anaguchi et al 2005** [22] | Patellar | *In vivo* and *in vitro* | Rabbit | TGF- β1 |
| **Anitua et al 2005** [28] | Semitendinosus | *In vitro* | Human | Autologous platelets-rich clots |
| **Aspenberg et al 2004** [35] | Achilles | *In vivo* and *in vitro* | Rat | Platelet concentrate injection |
| **Banes et al 1995** [51] | Flexor tendons | *In vitro* | Avian | PDGF-BB, IGF-I |
| **Bidder et al 2000** [67] | Flexor tendon | *In vitro* | Canine | AF, VEGF |
| **Boyer et al 2001** [82] | Flexor tendon | *In vitro* | Canine | VEGF |
| **Dahlgren et al 2005** [145] | Flexor digitorum superficialis | *In vitro* | Horse | IGF-I, TGF- β1 (temporal expression) |
| **Duffy et al 1995** [168] | Flexor tendon | *In vitro* | Canine | FGF |
| **Harwood et al 1999** [250] | flexor digitorum profundus | *In vitro* | Canine | BFGF, PDGF-BB |
| **Ngo et al 2001** [461] | Flexor digitorum profundus | *In vitro* | Rabbit | TGF- βr isoforms (RI,RII,RIII) (evaluation of distribution during tendon healing) |
| **Spindler et al 1996** [607] | Patellar and ACL | *In vitro* | Sheep | PDGF-AB, TGF- β1 |
| **Taylor et al 2002** [625] | Patellar | *In vivo* and *in vitro* | Rabbit | Autologous blood injection |
| **Thomopoulos et al 2005** [633] | Flexor tendon fibroblasts | *In vitro* | Canine | PDGF-BB, bFGF, VEGF, BMP-2 |
| **Yoshikawa et al 2001** [687] | Flexor tendon and peroneal tendon | *In vitro* | Rabbit | PDGF-BB |
| **Zhang et al 2004** [695] | Flexor tendon | *In vitro* | Rabbit | TGF-β neutralizing antibody (effect on TGF-β–induced collagen I production) |

**Table 1** (*continued*)

| Author | Tendon | Type of study | Model | Object of study |
|---|---|---|---|---|
| **Zhang et al 2003** [696] | Achilles tendon | *In vitro* | Rat | VEGF |
| **Dines et al 2007** [166] | Rotator Cuff | *In vivo* and *in vitro* | Rat | rhGDF-5 (applied to suture) |
| **Hamada et al 2006** [245] | Flexor tendon | *In vitro* and *in vivo* | Rabbit | bFGF (on nylon filament) |
| **Murray et al 2007** [453] | Supraspinatus | *In vivo* | Rat | CDMP2 |
| **Nakama et al 2006** [455] | Flexor Digitorum Profundus | *In vivo* | Rabbit | VEGF, VEGFR-1, CTGF |
| **Rodeo et al 2007** [539] | Infraspinatus tendon | *In vivo* | Sheep | Osteoinductive Growth Factors |
| **Rodeo et al 2007** [537] | Infraspinatus tendon | *In vivo* | Sheep | BMP-2, BMP-7, TGF-β1, TGF- β2 TGF- β3, BMP-12 |
| **Costa et al 2006** [136] | Flexor digitorum profundus tendons | *In vitro* | Rabbit | IGF-1, PDGF-BB, bFGF |
| **de Wit et al 2009** [155] | Digital flexor tendon | *In vivo* | Rabbit | Auto-cross linked hyaluronic acid gel |
| **Awad et al 1999** [42] | Patellar tendon | *In vivo* | Rabbit | Bone marrow MSCs suspended in type I collagen gel |
| **Awad et al 2003** [41] | Patellar tendon | *In vivo* | Rabbit | Collagen gels seeded with bone marrow-derived MSCs contracted onto sutures |
| **Juncosa-Melvin et al 2006** [292] | Achilles tendon | *In vivo* | Rabbit | Autogenous tissue-engineered constructs seeded with mesenchymal stem-cells |
| **Juncosa-Melvin et al 2007** [294] | Patellar tendon | *In vivo* | Rabbit | Collagen sponges seeded with mesenchymal stem cells |
| **Cao et al 2002** [100] | Feet flexor tendon | *In vivo* | Hen | Autologus tenocytes mixed with unwoven polyglycolic acid fibers wrapped with intestinal submucosa |
| **Cao et al 2006** [99] | Feet flexor tendon | *In vitro* | Hen | Tenocytes seeded on polyglicolic acid fibers |
| **Juncosa-Melvin et al 2006** [293] | Patellar tendon | *In vivo* | Rabbit | MSCs into a gel-sponge composite |
| **Liu et al 2006** [351] | Flexor digital superficial tendon | *In vivo* | Pig | Dermal fibroblasts seeded on polyglycolic acid unwoven fibers |
| **Ouyang et al 2003** [481] | Achilles tendon | *In vivo* | Rabbit | Knitted poly-lactide-co-glycolide loaded with bone marrow stromal cells |
| **Basile et al 2008** [60] | Flexor digitorum longus | *In vivo* | Mouse | rAAV-*Gdf5*-loaded freeze-dried tendon allografts |
| **Chong et al 2007** [120] | Achilles tendon | *In vivo* | Rabbit | Bone marrow-derived MSCs in a fibrin carrier |
| **Anitua et al 2005** [28] | *Semitendinosus* | *In vitro* | *Human* | PDGF and TGF-β1 |
| **Schnabel et al 2006** [569] | *Flexor digitorum superficialis* | *In vitro* | *Equine* | PRP (platelet rich plasma) |

growth factors may not be effective enough. Nevertheless, several animal studies have demonstrated beneficial results from local injection of growth factors [585].

The literature search, performed as described above, allowed to retrieve 46 studies on growth factors for tendon repair (Table 1).

*Insulin-like Growth Factor (IGF)*

Insulin-like growth factor (IGF) has anabolic effects on healing tendons by stimulating protein synthesis, increasing cell proliferation, collagen synthesis, and decreasing swelling [4,51,642]. IGF-I and -II increase collagen synthesis in a dose-dependent manner in animal models, and also increase proteoglycan synthesis [4,452]. The response to cytokines may be site-specific, and IGF-I induces a higher rate of collagen synthesis in rabbit flexor tendons compared to Achilles tendons [4]. IGF-I acts synergistically with Platelet Derived Growth Factor BB (PDGF) to stimulate tenocyte migration [4,51]. IGF-I and PDGF also act synergistically with cyclical loading, stimulating tenocytes both mitogenically and matrigenically [50]. Rats in the IGF-1 treated group had higher Achilles functional index scores and accelerated recovery compared to control groups [328]. Degenerate equine flexor tendons treated with local injections of IGF-I demonstrated decreased soft tissue swelling, increased DNA and collagen synthesis, improved echodensity of degenerate lesions and improved tendon biomechanics compared to control tendons injected with saline [146]. *In vitro* studies have shown that the addition of IGF-1 to tenocytes in culture induces matrix synthesis, but did not affect matrix turnover [328]. IGF-1 seeded scaffold exhibited better histology scores and a higher ultimate load-to-failure than those with the scaffold alone in a rat rotator cuff model [165,645]. IGF-I was more potent than insulin in stimulating protein synthesis and cell proliferation [5] and more potent than insulin-like growth factor-II (IGF-II) in stimulating increased cell proliferation [3]. However, it was less potent than IGF-II in increasing proteoglycan synthesis [3]. The two factors in combination did not enhance the synthesis of matrix proteins and DNA as compared with either factor alone [3]. IGF-I counteracted the decrease in collagen synthesis and stimulated protein synthesis to a higher degree than IGF-II in long-term culture [3]. Both factors had similar effects on matrix turnover [3]. When the effects of IGF-I were compared on various types of tendon segments [4], intrasynovial proximal segments synthesized 15 times less DNA than other tendon segments and IGF-I stimulated matrix and DNA synthesis of all tendon segments in a dose-dependent manner in intervals from 10 to 1,000 ng/mL [4].

*Transforming Growth Factor-β (TGF-β) and Bone Morphogenetic Protein (BMP)*

Transforming growth factor beta-1 (TGF-β 1) is a multipotent growth factor involved in wound healing and scar formation. During wound healing, TGF-β is released from degranulating platelets and secreted by all the major cell types participating in the healing process, including lymphocytes, macrophages, endothelial cells, smooth muscle cells,

epithelial cells, and fibroblasts [679]. Scar tissue formation has been closely associated with the presence of the 3 TGF-β isoforms (TBF-β1, 2, and 3). Although adult wounds heal with an abundance of scar tissue, which is correlated with increased expression of TGF-β1, fetal wounds heal without scar and without expression of TGF-β1. Therefore, inhibition of TGF-β1 or exogenous application of TGF-β3 may reduce scar tissue formation in the interface. TGF-β3 is expressed during fetal tendon development. Application of TGF-β1 coupled with suppression of TGF-β2 and -3 led to mechanically inferior tissue despite increased cross-sectional area. This suggests that, although TGF-β1 results in exuberant production of scar tissue at the repair site, this tissue is mechanically weaker than normal tissue [447]. TGF- β has also been shown to have a role in both tendon healing and adhesion formation [584]. Achilles tendons treated with TGF-β1-transfected BMSCs showed higher concentrations of collagen I protein, more rapid matrix remodelling, and larger fiber bundles [266]. Nevertheless TGF-β may be detrimental to tendons. Increased levels of TGF-β have been reported in tendinopathic human Achilles tendons, and in rabbit flexor tendons after injury [110, 184]. TGF-β induces increased collagen production in rabbit tenocytes, and upregulation of TGF-β receptors occurs following flexor tendon injury and in tendinopathic human Achilles tendons [184, 309, 461]. TGF-β results in scar formation and fibrosis, and TGF-β 1 expression is increased in patients with postburn hypertrophic scarring and keloids [225, 502]. In this situation, inhibition of growth factor action can provide beneficial results. A single dose of antibody to TGF-β1 at the time of flexor tendon repair in rabbits counteracted the negative effect of scar formation and fibrosis. The resultant decrease in adhesion formation produces an increased range of motion after surgery compared to control specimens [111].

In 1994, two groups of researchers reported the discovery of proteins involved in the formation of the mammalian skeleton [112, 615]. Subsequently, the human equivalents of these proteins were identified, and have been termed bone morphogenetic proteins (BMPs) [626]. Bone morphogenetic proteins are members of the TGF-b superfamily, and play an important role during embryogenesis and tissue repair in postnatal life [56, 233, 487, 680]. BMP-12 (also known as growth and differentiation factor 7) and BMP-13 (growth and differentiation factor 6) are both expressed at the embryonic development sites that form tendons and their insertions [679]. These molecules are distinct from the osteoinductive BMPs (BMP-2,-4,-7), and induce formation of tendon and fibrocartilage. Studies have reported that administration of recombinant human BMP-12 (rhBMP-12) and rhBMP-13 leads to induction of neotendon/ligament formation in rats and improved healing of tendon laceration [679]. BMP-2, BMP-7, BMP-12, TGF-β1, TGF-β2, TGF-β3 and fibroblast growth factor (FGF) improved formation of new bone and fibrocartilage at the healing tendon attachment site, resulting in improved load to failure [537]. BMP-12 determined increased formation of new bone and fibrocartilage at the healing tendon attachment site in sheep infraspinatus repair model, and biomechanical testing showed improved load-to-failure [323].

*Cartilage Derived Morphogenetic Protein growth factor (CDMP)*

Cartilage Derived Morphogenetic Protein growth factor (CDMP) -1, -2 and -3, are equivalent to human BMP 14, 13 and 12 [191]. Their injection into lacerated rat Achilles tendons resulted in a significant dose-related increase in strength and stiffness [191]. CDMP-2 injection into transected rabbit Achilles tendons resulted in a 35% increase in mechanical strength 14 days postoperatively compared to controls [190]. CDMP-2–treated repairs of supraspinatus tendon tears were significantly stronger than the untreated repairs and histological analysis showed more organized healing [453].

*Platelet Derived Growth Factor (PDGF)*

Platelet Derived Growth Factor (PDGF) and Epidermal Growth Factor (EGF) were the first two growth factors applied *in vitro* to study the effect on tendon healing resulting in stimulation of tendon fibroblast proliferation [585].

PDGF-BB (PDGF composed of two B (-BB) chains) acts as a mitogen and chemotactic cytokine that can potentially enhance ligament and tendon healing, but improved healing with PDGF is dependent on the dosage, timing, and delivery vehicle used. In a dog model PDGF-BB stimulated cell activity [632] and increased proximal interphalangeal joint rotation and tendon excursion, but did not determine improvements in tensile properties [632]. *In vitro* PDGF-BB, as IGF-I, also has a fundamental role, in addition to mechanical load, to stimulate DNA synthesis [51]and, as FGF, to increase integrin expression by intrasynovial flexor tendon cells [632]. PDGF-BB stimulated matrix and DNA synthesis of intrasynovial intermediate and proximal segments of deep flexor tendons, and extrasynovial peroneal tendons of rabbits in a dose-dependent manner in the range from 0.1 to 100 ng/mL [687]. PDGF-BB stimulated collagen synthesis and noncollagen protein synthesis in proximal intrasynovial tendon segments more than in extrasynovial peroneal tendon segments, and DNA synthesis less in proximal than in intermediate intrasynovial tendons. However, the estimated maximal stimulation $E_{max}$ by PDGF-BB were similar in the three types of tendon segments [687].

*Preparations Rich in Growth Factor (PRGF)*

There are currently several commercially available systems to produce a ‘‘platelet-rich plasma’’ or ‘‘platelet gel’’ from autologous blood. These systems involve spinning autologous blood in a centrifuge to form a dense, suturable fibrin matrix that can be easily placed directly at the tendon repair site. One technical problem with these systems is that many use human or bovine thrombin to form the platelet-rich plasma. Excess thrombin causes premature platelet activation and degranulation, causing immediate release of the platelet-derived cytokines. Newer systems have omitted the use of thrombin to prevent this phenomenon during processing. Currently, there are no clinical studies on the efficacy of this treatment though theoretically it holds promise. Proteins released from platelet-rich

clots have potential beneficial effect on tendon healing. PRGF stimulated VEGF synthesis in tenocytes, which also exhibited a different pattern of HGF production, and enhanced hyaluronic acid (HA) synthesis; platelet-secreted TGF-β may be involved in HA, but not in type I procollagen synthesis. Therefore, the biological effects of PRGF may depend on concentration of platelets and on the anatomical source of the cells [29]. Cultured tendon cells synthesised significantly higher VEGF and HGF levels in the presence of platelet-rich plasma (PRP)-clots, than in the presence of platelet-poor plasma(PPP)-clots [28].

Equine flexor digitorum superficialis tendon explants cultured in media consisting of PRP showed enhanced gene expression of the matrix molecules COL1A1, COL3A1, and COMP with no concomitant increase in the catabolic molecules MMP-3 and MMP-13, compared to all other blood products tested [569].

From a clinical point of view, in a pilot non-randomized single group study of 14 patients, autologous PRP for arthroscopic RC repair provided good clinical results [521]. A recent randomized controlled trial in patients with chronic Achilles tendinopathy showed no advantages of a PRP injection compared with a saline injection. On the other hand, data from another recent randomized controlled trial showed that treatment of patients with chronic lateral epicondylitis with PRP reduces pain and significantly increases function, exceeding the effect of corticosteroid injection [499]. In a randomised controlled trial, exogenous application of platelet-leukocyte gel during open subacromial decompression contributed to improved patient outcome (recovery was faster and patients returned earlier to daily activities and also took less pain medication than control subjects) [179].

*Interleukin-10*

Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine, shown to inhibit scar formation in fetal wound healing. The role of IL-10 in adult tendon healing and scar formation, however, remains unknown [581]. Over-expression of IL-10 after injection of the lentiviral vector, increased maximum stress in patellar tendon and percent relaxation [533].

*Recombinant human osteogenic protein-1 (rhOP-1)*

Recombinant human osteogenic protein-1 (rhOP-1) stimulates the proliferation of tendon cells and their ability to synthesize and accumulate proteoglycans (PGs) and collagen in their extracellular matrix. The addition of rhOP-1 to cell culture media resulted in significant increases in cell proliferation, DNA content, and the synthesis of PGs and collagen, compared to control cultures [683].

*Vascular Endothelial Growth Factor (VEGF)*

Vascular endothelial growth factor (VEGF) is an endothelial mitogen which promotes angiogenesis and increases capillary permeability [185]. VEGF-induced vasodilatation results partly through stimulation of nitric oxide synthase in endothelial cells [685]. VEGF is

expressed in ruptured and foetal human Achilles tendons, but not in normal adult Achilles tendons [517] and is expressed in high concentration in healing flexor tendons 7 to 10 days following repair, with a return to normal by 14 days [682].

VEGF plays a key role in tendon healing. In a canine flexor tendon repair model, the expression of VEGF mRNA was increased at the repair site seven days post-operatively, with peak levels occurring 10 days after surgery [67,82].

In a repairing tendon there is a gradient of cell populations expressing VEGF: the majority of cells within the repair site itself express VEGF mRNA. However, minimal levels accumulate within cells of the epitenon. By contrast, expression of type alfa l (I) collagen and histone H4 does not differ significantly between the epitenon and the repair site [67].

VEGF treatment at the time of surgical repair of transacted rat Achilles tendons resulted in significantly improved tensile strength at two weeks, when the plantaris tendon was preserved [696]. However, by four weeks, no significant difference was present.

VEGF injected into repaired Achilles tendons in a rat model determined improved tensile strength early in the course of healing [696]. In contrast, a recent study on the ability of VEGF on graft healing in a sheep ACL reconstruction model showed detrimental effects: although there was increased vascularity, the stiffness of the femur–graft–tibia complex was significantly lower than in controls [686].

The administration of exogenous VEGF can significantly improve tensile strength early in the course of the rat Achilles tendon healing and was associated with increased expression of TGF-β [696].

VEGF, VEGFR-1, and CTGF cell densities were increased in the Flexor Digitorum Profundus (FDP) tendon at the epicondyle of rabbits loaded limb compared to the unloaded limb [455].

*Fibroblast Growth Factor (FGF)*

Intrasynovial flexor tendon cells revealed increased expression of integrins when exposed to either basic fibroblast growth factor or platelet-derived growth factor-BB over a wide range of growth factor concentrations [245].

The epitenon of rabbit tendon showed a vigorous fibroblastic response to thread coated with bFGF and the ultimate load also was increased significantly at 3 weeks after surgery [245].

*Recombinant Human Growth Differentiation Factor (rhGDF)*

Rats supraspinatus tendons repaired with recombinant human growth differentiation factor (rhGDF)-5-coated sutures showed significantly higher ultimate tensile load and stiffness compared with control sutures, significant tendon hypertrophy at 3 weeks, but there were no significant differences at 6 weeks.

### *2. Gene therapy*

Gene therapy delivers genetic material (DNA) to cells using viral or non-viral vectors or direct gene transfer. Growth factors have the potential to enhance native repair responses in tendon and ligamentous lesions. However, methods to apply growth factors to the site of injury for extended period are lacking [223]. The transfer of genes which encode healing factors is a challenging solution to this problem. Growth factors, in addition to direct application, can be delivered to the tendon also by gene therapy, as it carries genes encoding growth factors rather than growth factors directly [268, 479, 664]. The cells incorporate the genetic material, and begin to produce growth factors (Fig.1). In this way, the exposure to growth factors is more prolonged. The vectors most frequently used are adenovirus, adeno-associated virus, cationic liposomes, and haemagglutinating virus of Japan-liposomes complexes [268, 479, 664]. Non-viral vectors are less pathogenic, but also less efficient. Viral vectors, in fact, allow the insertion of genes into cells that have ceased to live. This is important in tendons, as tenocytes not divide actively [585]. Potential complications associated with the use of vectors are loss of transgene expression and scarring and adhesion formation secondary to inflammation. Scarring and adhesion formation is potentially most troublesome when encountered in flexor tendons. Liposome-plasmid vectors, adenovirus, and adeno-associated virus elicit a less severe tissue reaction compared to that normally seen in the early inflammatory phase of tendon injury. The viral vectors have been suggested to cause less tissue reaction than the liposome-plasmid complex, and adeno-associated virus in particular caused virtually no response in the endotenon. Other possible techniques for gene transfection are the direct injection into the arterial circulation of liposomes and DNA electrotransfer, which involves the application of an electrical field after the injection of naked DNA into tissues.

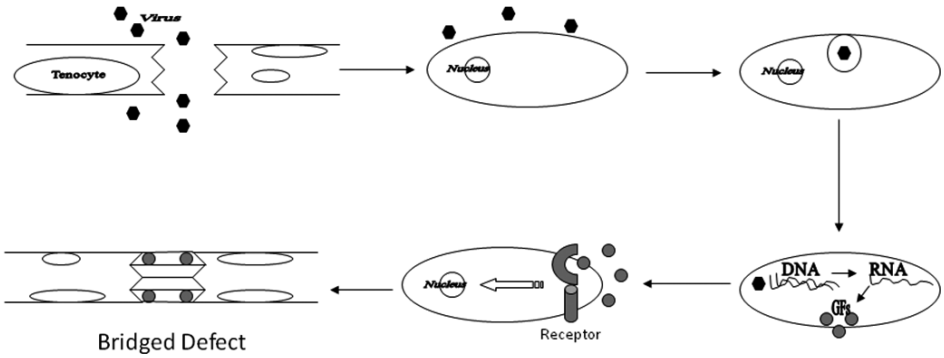


**Figure 1:** A. Virus encoding for growth factor (GF) delivering to the tendon injury site. B. Virus binding to the wall of the tenocyte. C. Virus entering the cytoplasm. D. Transription of viral DNA to RNA and translation to growth factor. E. Release of growth factor from the tenocyte into the surrounding environment and its binding to receptors on sourrounding tenocytes. F. Tendon healing.

Gene transfer using vectors can be achieved via an *in vivo* or *ex vivo* technique. *In vivo* transfer involves direct application of the gene to the relevant tissue. In the *ex vivo* technique, target cells are first removed from the body, before gene transfer is performed in the laboratory. Once successful transfection is achieved, the cells are transferred back into the body. *In vivo* transfection is less invasive and technically easier, and treatment can be commenced during the acute phase of injury. The disadvantage of *in vivo* transfer is non-specific infection of cells adjacent to the site of injury. Furthermore, the success of gene transfer cannot be confirmed, and, in areas of relative cell paucity, only a few cells may be transfected. The use of highly transgenic vectors and injection into areas with a high concentration of cells will ensure transfection of a large proportion of cells. More time is required for *ex vivo* transfection, but this technique avoids the complication of non-specific transfection, allows successful transfection to be confirmed, and also allows *in vitro* expansion of cells if required [585].

The literature search, performed as described above, allowed to retrieve 18 studies on gene therapy for tendon repair (Table 2).

**Table 2:** Gene Therapy

| Author | Tendon | Type of study | Model | Object of study |
|---|---|---|---|---|
| **Gerich et al 1997** [223] | Semitendinosus and patellar tendons | *In vivo* | Rabbit | **Retroviral MFG lacZ, BAG lacZ neo, adenoviral LacZ** |
| **Nakamura et al 1996** [456] | Patellar tendon | *In vivo* and *in vitro* | Rat | **HVJ-liposome (containing platelet-derived growth factor (PDGF)-B cDNA)** |
| **Bolt et al 2007** [78] | Achilles tendon | *In vivo* and *in vitro* | Rat | **adenovirus-mediated transgene expression of BMP-14** |
| **Majewski et al 2008** [411] | Achilles tendon | *In vivo* and *in vitro* | Rat | **Adenovirus-mediated transgene expression of BMP-12** |
| **Mehta et al 2005** [427] | Flexor tendon | *In vivo* and *in vitro* | Rabbit | **Adenovirus-mediated transgene expression of BMP-13** |
| **Rickert et al 2005** [534] | Achilles tendon | *In vivo* and *in vitro* | Rat | **Adenovirus-mediated transgene expression of GDF-5** |
| **Shimomoura et al 2003** [589] | Patellar tendon | *In vitro* | Human | **Antisense oligonucleotide that selectively target the type V procollagen $\alpha$1 chain Mrna** |
| **Wang et al 2005** [668] | Intrasynovial tendons tenocytes | *In vitro* | Rat | **Adenoassociated viral vectors containing exogenous bFGF gene** |
| **Wang et al 2005** [667] | Intrasynovial tendons tenocytes | *In vitro* | Rat | **Tenocytes modified with VEGF gene compared with tenocytes modified with exogenous PDGF gene** |
| **Wang et al 2004** [666] | Intrasynovial tendons tenocytes | *In vitro* | Rat | **Plasmid containing PDGF c-DNA with liposome** |
| **Zhu et al 2006** [698] | Digitorum Profundus Tendons | *In vivo* and *in vitro* | Rabbit | **Adenoviral, adenoassociated viral (AAV), and liposome–plasmid vectors** |

**Table 2** (*continued*)

| Author | Tendon | Type of study | Model | Object of study |
|---|---|---|---|---|
| **Lattermann et al 2004** [330] | Flexor digitorum longus tendon | *In vivo* | Rabbit | **Adenoviral vector carrying the luciferase marker gene vs adenoviral vector injected into the bone trough** |
| **Lou et al 2001** [380] | Tendon cells | *In vitro* | Chicken | **Adenovirus mediated BMP-12 gene transfer** |
| **Jayankura et al 2003** [284] | 1)Achilles tendons 2) Patellar tendons | *In vivo* and *in vitro* | 1)Rat and mouse 2)Rabbit | **Plasmid carrying the *lacZ* marker gene** |
| **Mi et al 2000** [436] | Patellar tendon | *In vivo* and *in vitro* | Rabbit | **Adenovirus-mediated intra-articular gene transfer of TGF-β1** |
| **Pelinkovic et al 2003** [500] | Supraspinatus tendon | *In vitro* | Rat | **Genetically engineered muscle-derived cells** |
| **Rickert et al 2005** [534] | Achilles tendon | *In vitro* and *in vivo* | Rat | **Adenoviral GDF-5 transfer** |
| **Ozkan et al 1999** [486] | **Patellar tendon** | ***In vitro* and *in vivo*** | **Rat** | **HVJ-liposome complexes containing beta-galactosidase cDNA** |

*Adenovirus and Adeno-Associated Virus*

The adenovirus was the most effective vector in short-term experiments. However, expression was transient. Although retrovirus gave lower initial transduction efficiencies, the percentage of transduced cells could be increased using the selectable marker gene neo [223]. In an *in vivo* marker study, Gerich et al injected adenovirus into the rabbit patellar tendon [223]. Transduced cells could be observed preferentially in the subsynovial layer at a declining frequency over a 6-week period. The allogenetic transplantation of *in vitro* retrovirally transduced fibroblasts into the patellar tendon resulted in a greater number of transduced cells. Although the number of lacZ(+) cells declined with time, positive cells were still present 6 weeks after transplantation. Furthermore, the transplanted cells, unlike cells transduced *in situ* with adenovirus, migrated from the injection site and integrated into the crimp of the tendon. Mehta et al [427] determined that adenovirus-based gene therapy is an efficient means of gene delivery to rabbit flexor tendons, but the transduction efficiency of transgenes was dose dependent across the tested titers. However, adenovirus-induced inflammation was notable only at the highest titer [427].

Growth and differentiation factor-5 (GDF-5) induces tendon tissue and stimulates tendon healing. Rickert et al [534] injected adenovirus particles into transected Achilles tendons of rats. *In vitro*, GDF-5 was secreted with a peak after 2 weeks. *In vivo*, GDF-5 transgene expression showed a peak at 4 weeks. At 8 weeks, GDF-5 specimens were thicker with a trend to higher strength. Histology showed greater cartilage formation in type II collagen stains than in controls.

Basic fibroblast growth factor (bFGF) promotes collagen production in healing tendons. Wang et al [668] transferred the exogenous bFGF gene to proliferating tenocytes by adeno-

associated viral (AAV) vectors and investigated its effects on the expression of the collagen genes in an *in vitro* tenocyte model from explant cultures of rat intrasynovial tendons. Positive β-galactosidase staining confirmed the effectiveness of AAV2-mediated gene delivery to tenocytes. The level of expression of the bFGF gene increased significantly after gene transfer. Levels of expression of type I and III collagen genes after transfer of the exogenous bFGF gene were increased significantly compared with those in the cells treated with sham vectors or in non-treatment controls.

Adenovirus-mediated *in vitro* BMP-12 gene transfer into chicken tendon cells increased type I collagen synthesis, and *in vivo* resulted in a two-fold increase of tensile strength and stiffness of repaired tendons[380].

Lattermann et al[330] showed that the bone canal provides a more efficient target for direct adenoviral gene delivery than the tendon and demonstrated the feasibility of the bone trough immersion technique, since sustained gene expression within the tendon-bone interface was obtained for up to 4 weeks.

*Haemagglutinating virus of Japan (HVJ)*

The rate of transfection of a reporter gene in rat patellar tendon using the haemagglutinating virus of Japan (HVJ) liposome-mediated gene transfer method by intra-arterial delivery was significantly greater than controls[486]. Nakamura et al[457] injected directly into the injured patellar ligament of rats a HVJ-liposome suspension containing PDGF-B cDNA. PDGF-B gene transfer caused the enhanced expression of PDGF in healing ligament with an initial promotion of angiogenesis and enhanced collagen deposition in the wound.

Gene therapy with BMPs may improve the healing ability of injured musculoskeletal tissues. Achilles tendon transduced with BMP-14 exhibited less visible gapping, a greater number of neotenocytes at the site of healing, and 70% greater tensile strength than did either those transduced with GFP or the sham controls at two weeks after repair[78].

The maximum failure load of healing Achilles tendons was significantly increased when the tendon was managed with BMP-12, and the tendon stiffness was significantly higher at 1, 2 and 4 weeks[411]. Moreover, the size of the rupture callus was increased in the presence of BMP-12 and there was evidence of accelerated remodelling of the lesion in response to BMP-12[411].

Majewski et al[411] evaluated the histological and biomechanical effects of BMP-12 gene transfer on the healing of rat Achilles tendons using a genetically modified muscle flap. Biopsies of autologous skeletal muscle were transduced with a type-five, first-generation adenovirus carrying the human BMP-12 cDNA (Ad.BMP-12) and surgically implanted around experimentally transected Achilles tendons in a rat model. The authors concluded that treatment with BMP-12 cDNA-transduced muscle grafts produced a promising acceleration and improvement of tendon healing, particularly influencing early tissue

regeneration, leading to quicker recovery and improved biomechanical properties of the Achilles tendon.

*Antisense oligonucleotide*

Type V collagen plays a role in regulating the diameter of type I collagen fibrils; reducing its level may lead to the formation of larger collagen fibrils in healing ligaments. Hence, type V collagen antisense gene therapy may be an approach to achieve this goal. Shimomura et al [589] hypothesized that antisense oligonucleotides that selectively target the type V procollagen $\alpha$1 chain mRNA could partially reduce the synthesis of type V procollagen $\alpha$1 chain in human tenocytes. Western blotting showed that antisense oligonucleotides (AS-V1 and AS-V2) significantly reduced the synthesis of type V procollagen $\alpha$1 chain. In addition, the reverse transcription polymerase chain reaction showed that both antisense oligonucleotides partially reduced type V procollagen $\alpha$1 chain mRNA expression.

*Plasmids*

A plasmid carrying the *lacZ* marker gene was injected into the Achilles tendons of rat and mouse, and into the patellar tendons of rabbit by Jayankura et al [284]. At 48 hours, transduced cells were found in the injected zones of the tendons but represented a minority of the tendon cells. A kinetics study in rats permitted observation of a gradual decrease with time in the $\beta$-gal-expressing cell number; at day 42, gene expression was no longer detected. No inflammatory reaction was observed [284].

Wang et al [666] transferred using a plasmid containing the PDGF complementary deoxyribonucleic acid (cDNA) with liposome the PDGF-B gene to tenocytes obtained from explant cultures of rat intrasynovial tendons, and investigated its effects on the expression of the PDGF gene and the type I collagen gene in an *in vitro* tenocyte culture model. Reverse transcription polymerase chain reactions (RT-PCR) assessed the enhancement of the expression of the PDGF gene, the efficiency of the gene transfer, confirmed by the presence of exogenous PDGF cDNA in the tenocytes. Quantitative analysis of the products of RT-PCR showed significant increased levels of expression of the type I collagen gene by tenocytes.

The tissue reactions of adenoviral, adenoassociated viral (AAV), and liposome–plasmid vectors in tendons was compared with the healing responses of injured flexor tendons of rabbits [698]. The tissue reactions of the liposome–plasmid vector in tendons were the most prominent among the 3 vectors tested. The adenoviral vector elicited a moderate degree of tissue reaction. The AAV2 vector caused remarkable reactions in epitenon but almost no reactions in endotenon. The 3 gene delivery systems tested elicit less severe tissue reactions in flexor tendons compared with early-stage inflammatory changes in injured tendons. Adenoviral and AAV vectors elicit less severe tissue reactions than liposome–plasmid vectors. The AAV2 vector appears to cause almost no reaction in the endotenon.

### *3. Tissue engineering with mesenchymal stem cells*

Technologic advances in biology and engineering have resulted in marked improvements in the design and manufacture of tissue-engineered substitutes that can modify and maintain living tissue [268, 479, 664]. Tissue engineering is an emerging field made up of the combination of scaffold, cell and stimulation or their stand-alone application [45, 46]. Mesenchymal stem cells (MSC) are capable to differentiate into a variety of specialized mesenchymal tissues including bone, tendon, cartilage, muscle, ligament, fat, and marrow stroma [45, 46] (Fig.2). Tissue engineering can be divided into 2 subtypes: the *in vivo* approach and the *ex vivo*, *de novo* one [268, 479, 664]. The *in vivo* approach permits the self-regeneration of small tissue lesions. The *ex vivo*, *de novo* approach is designed to produce functional tissue that can be implanted in the body [585]. Tissue engineering is a multidisciplinary field founded on three fundamental principles: the use of healthy multipotent cells that are nonimmunogenic, easy to isolate, and highly responsive to distinct environmental cues; (2) the development of carrier scaffolds that provide short-term mechanical stability of the transplant and a template for spatial growth of the regenerate tissue; and (3) the delivery of growth factors that drive the process of cell differentiation and maturation [268, 479, 664].

The literature search, performed as described above allowed to retrieve 20 studies on MSC for tendon repair (Table 3). MSC can be applied directly to the site of injury or can

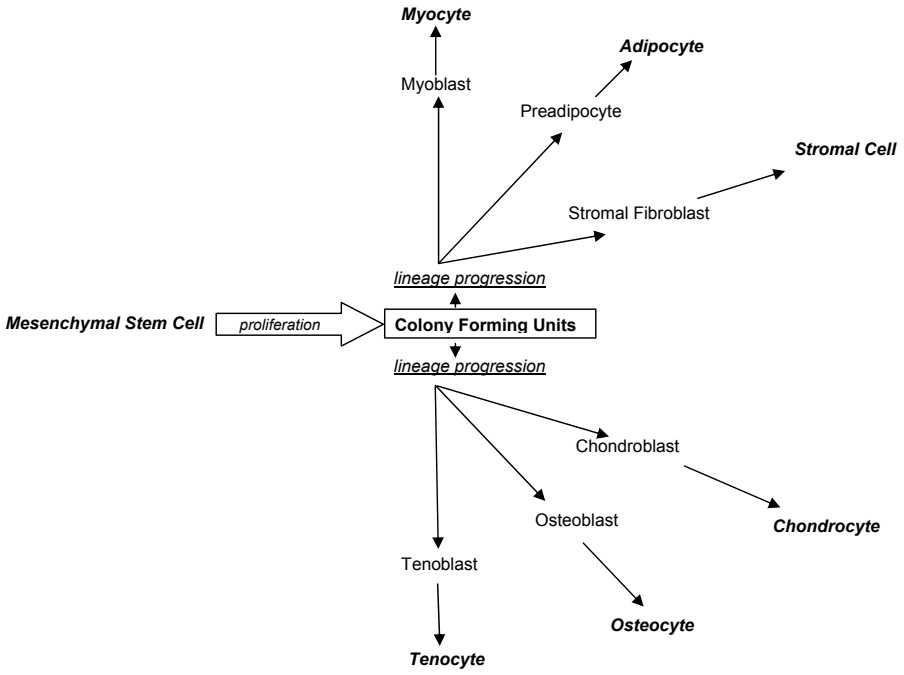


**Figure 2:** Schematic representation of mesenchymal stem cell differentiation.

**Table 3:** Tissue engineering

| Author | Tendon | Type of Study | Model | Object of study |
|---|---|---|---|---|
| **Omae et al 2009** [475] | Infraspinatus tendon | *In vitro* | Dog | Decellularized multilayer tendon slices seeded with bone marrow stromal cells. |
| **Young et al 1998** [689] | Achilles Tendon | *In vitro* | Rabbit | Marrow derived MSCs suspended in a collagen gel delivery vehicle |
| **Schnabel et al 2009** [568] | Flexor digitorum superficialis | *In vitro* | Horse | MSCs and IGF-I genes enhanced mesenchymal stem cells |
| **Funakoshi et al 2005** [204] | Infraspinatus | *In vitro* | Rabbit | Chitosan-based hyaluronan hybrid scaffold with seeded fibroblasts |
| **de Wit et al 2009** [155] | Digital flexor tendon | *In vivo* | Rabbit | Auto-cross linked hyaluronic acid gel |
| **Awad et al 1999** [42] | Patellar tendon | *In vitro* and *in vivo* | Rabbit | Bone marrow MSCs suspended in type I collagen gel |
| **Awad et al 2003** [41] | Patellar tendon | *In vitro* and *in vivo* | Rabbit | Collagen gels seeded with bone marrow-derived MSCs contracted onto sutures |
| **Juncosa-Melvin et al 2006** [292] | Achilles tendon | *In vitro* and *in vivo* | Rabbit | Autogenous tissue-engineered constructs seeded with mesenchymal stem-cells |
| **Juncosa-Melvin et al 2007** [294] | Patellar tendon | *In vitro* | Rabbit | Collagen sponges seeded with mesenchymal stem cells |
| **Cao et al 2002** [100] | Feet flexor tendon | *In vitro* and *in vivo* | Hen | Autologus tenocytes mixed with unwoven polyglycolic acid fibers wrapped with intestinal submucosa |
| **Cao et al 2006** [99] | Feet flexor tendon | *In vitro* | Hen | Tenocytes seeded on polyglicolic acid fibers |
| **Juncosa-Melvin et al 2006** [293] | Patellar tendon | *In vitro* and *in vivo* | Rabbit | MSCs into a gel-sponge composite |
| **Liu et al 2006** [351] | Flexor digital superficial tendon | *In vitro* and *in vivo* | Pig | Dermal fibroblasts seeded on polyglycolic acid unwoven fibers |
| **Ouyang et al 2003** [481] | Achilles tendon | *In vitro* and *in vivo* | Rabbit | Knitted poly-lactide-co-glycolide loaded with bone marrow stromal cells |
| **Basile et al 2008** [60] | Flexor digitorum longus | *In vitro* | Mouse | rAAV-Gdf5-loaded freeze-dried tendon allografts |
| **Chong et al 2007** [120] | Achilles tendon | *In vitro* and *in vivo* | Rabbit | Bone marrow-derived MSCs in a fibrin carrier |
| **Soon et al 2007** [599] | Achilles tendon | *In vitro* and *in vivo* | Rabbit | MSCs in a fibrin glue carrier |
| **Ju et al 2008** [291] | Achilles tendon | *In vivo* | Rat | Synovial MSCs |
| **Awad et al** [41] **2003** | Patellar tendon | *In vivo* | Rabbit | MSC–collagen graft |
| **Majima et al** [412] **2005** | Patellar tendon | *In vitro* | Rabbit | Fibroblasts seeded alginate-based chitosan hybrid polymer fibers |

be delivered on a suitable carrier matrix, which functions as a scaffold while tissue repair takes place [581-584]. The ideal scaffold for tendon engineering would possess the basic structure of the tendon, native extracellular matrix, and capability of cell seeding [475].

Decellularized multilayer tendon slices were seeded with bone marrow stromal cells (BMSC), harvesting BMSC and infraspinatus tendons from dogs. Histology showed the alignment of the seeded cells between the collagen fibers of the tendon slices. qRT-PCR analysis showed higher tenomodulin and MMP13 expression and lower collagen type I expression in the composite than in the BMSC before seeding, suggesting that BMSC might express a tendon phenotype in this environment [475].

Delivering mesenchymal stem cell-contracted, organized collagen implants applied to large tendon defects can significantly improve the biomechanics, structure, and probably the function of the tendon after injury [689]. A tissue prosthesis was implanted made up of cultured, autologous, marrow derived MSCs suspended in a collagen gel delivery vehicle and contracted onto a pretensioned suture, into a 1 cm long gap defect in a rabbit Achilles tendon [689]. Load related structural and material properties evaluated 4, 8 and 12 weeks later were greater than in the control repairs, which contained suture alone with natural cell recruitment. Furthermore, the treated tissue showed a significantly larger cross-sectional area, and their collagene fibers appeared to be better aligned then those in the controls.

The use of MSCs to enhance allograft osteointegration is a novel method offering the potential of more physiologic and earlier healing [599]. MSCs derived from synovium have a higher proliferation and differentiation potential than the other MSCs. Their potential to accelerate the early remodelling of tendon-bone healing histologically by producing more collagen fibers at 1 week and forming more oblique collagen fibers connecting the bone to tendon resembling Sharpey's fibers at 2 weeks has been shown [291]. Moreover, MSCs do not interfere with tendon-bone healing at 4 weeks [291].

MSCs have been investigated in the management of tendinopathy. MSCs and IGF-I genes enhanced MSCs (AdIGF-MSCs) on the healing of a collagenase-induced bilateral tendinopathy lesions in an equine flexor digitorum superficialis injury model. Both MSC and AdIGF-MSC injection resulted in significantly improved tendon histological scores [568].

Tissue engineering techniques using novel scaffold materials offer potential alternatives for managing irreparable rotator cuff tears.

A chitosan-based hyaluronan hybrid scaffold with seeded fibroblasts to repair infraspinatus tendons defects produced in rabbits, demonstrating an enhanced type I collagen production and a significant improvement in tensile strength and tangent modulus from 4 to 12 weeks post-operatively [204].

*In vivo*, the effect of auto-cross linked hyaluronic acid gel on adhesions and healing of injured and surgically repaired rabbit digital flexor tendons was studied, demonstrating a significantly faster increase in breaking strength with an accelerated tissue repair response after injury, but unaffected adhesions formation [155].

In rabbit, MSCs expanded in culture, suspended in type I collagen gel, and implanted into a surgically induced defect in the donor's right patellar tendon demonstrated significant increases in maximum stress, modulus, and strain energy density [42].

Changes in nuclear morphology of the MSCs in response to physical constraints provided by the contracted collagen fibrils may trigger differentiation pathways toward the fibroblastic lineage and influence the cell synthetic activity [41]. Controlling the contraction and organization of the cells and matrix will be critical to successfully produce tissue engineered grafts. Seeded collagen gels with rabbit bone marrow-derived MSCs and contracted onto sutures were implanted into full thickness, full length, central defects in the patellar tendons of the animals [41]. Repair tissues containing the MSC–collagen composites showed significantly higher maximum stresses and moduli than natural repair tissues at 12 and 26 weeks postsurgery [41].

Autogenous tissue-engineered constructs were fabricated in culture between posts in the wells of silicone dishes [294]. Constructs were implanted in bilateral 2 cm long gap defects in the rabbit's lateral Achilles tendon. At 12 weeks after surgery, no significant improvement were observed in any structural or mechanical properties or in histological appearance compared with control. The same authors tried also to determine how a tensile stimulus affects the gene expression of stem cell–collagen sponge constructs used to repair rabbit central patellar tendon defects [294].

MSCs were introduced into a gel-sponge composite showing cellular alignment comparable to that of normal tendon [293].

Cao et al [100] tested the feasibility of engineering tendon tissues with autologous tenocytes to bridge a tendon defect in either a tendon sheath open model or a partial open model in the hen. Flexor digitorum profundus defects were bridged either with a cell-scaffold construct in the experimental group or with scaffold material alone in the control group. At 14 weeks, the engineered tendons resembled the natural tendons grossly in both colour and texture, and displayed a typical tendon structure hardly distinguishable from that of normal tendons. The same authors also explored the feasibility of *in vitro* tendon engineering using the same type of cells and scaffold material [99]. Unwoven PGA fibres were arranged into a cord-like construct and fixed on a U-shape spring, and tenocytes were then seeded on PGA fibres to generate a cell-PGA construct. The results showed that tendon tissue could be generated during *in vitro* culture. In addition, the tissue structure and mechanical property became more mature and stronger with the increase of culture time.

Alginate-based chitosan hybrid polymer fibers showed much improved adhesion capacity with tenocytes compared with alginate polymer fiber [412]. The rAAV-*Gdf5* vector significantly accelerates wound healing in an *in vitro* fibroblast scratch model and, when loaded onto freeze-dried FDL tendon allografts, improves the metatarsophalangeal (MTP) joint flexion to a significantly greater extent than the rAAV*lacZ* controls do [60]. In an

experimental study on rabbits, a sharp complete midsubstance transection of the Achilles tendon was immediately repaired using a modified Kessler suture and a running epitendinous suture. Both limbs were used, and each side was randomized to receive either bone marrow-derived MSCs in a fibrin carrier or fibrin carrier alone (control). At six and 12 weeks, there were no differences between the groups with regard to morphometric nuclear parameters. Biomechanical testing showed improved modulus in the treatment group as compared with the control group at three weeks ($p < 0.05$) but not at subsequent time-periods [120].

Costa et al [136] tried to optimize tenocyte proliferation in 3 tendon cell populations using growth factor supplementation. They isolated cells of the synovial sheath, epitenon, and endotenon from rabbit flexor digitorum profundus tendons and maintained in culture. For all 3 tendon cell populations, proliferation at 72 h was greater in the presence of individual growth factors as compared to controls. In addition, a synergistic effect was observed. The combination of growth factors resulted in greater proliferation as compared to maximal doses of individual growth factors.

Synthetic oligo[poly(ethylene glycol)fumarate] (OPF)-based biomaterials were tested as a means to deliver fibroblasts to promote regeneration of central/partial defects in tendons and ligaments. To further modulate the swelling and degradative characteristics of OPF-based hydrogels, OPF crosslinking via a radically initiated, mixed-mode reaction involving poly(ethylene glycol) (PEG)-diacrylate and PEG-dithiol was investigated. After encapsulation, tendon/ligament fibroblasts remained largely viable over 8 days of static culture. While the presence of PEG-dithiol did not significantly affect cellularity or collagen production within the constructs over this time period, image analysis revealed that the 20% PEG-dithiol gels did appear to promote cell clustering, with greater values for aggregate area observed by day [85].

The use of a poly(ethylene glycol) diacrylate (PEGDA) hydrogel incorporated with hydroxyapatite (HA) and the cell-adhesion peptide RGD (Arg-Gly-Asp) was tested as a material for determining an *in vitro* tissue interface to engineer intact ligaments. Incorporation of HA into PEG hydrogels reduced the swelling ratio but increased mechanical strength and stiffness of the hydrogels. Further, HA addition increased the capacity for cell growth and interface formation. RGD incorporation increased the swelling ratio but decreased mechanical strength and stiffness of the material [498].

A novel fabrication system for photopatterning and assembling cell-laden oligo(polyethylene glycol)-fumarate: poly(ethylene glycol)-diacrylate (OPF:PEG-DA) hydrogels with high spatial fidelity and thickness using a controlled, inert nitrogen environment was described [247]. Cross-linking was performed using Irgacure-2959 photoinitiator and 365-nm light (~7 mW/cm2) to form gels ranging from 0.9 - 3 mm in width. Employing a N2 environment increased gel thickness up to 240%, generating gels greater than 1 mm thick prior to swelling. This technique was further applied for spatially controlled patterning of

primary tendon/ligament fibroblasts and marrow stromal cells in a single 1.5-mm thick laminated hydrogel construct. Cells encapsulated using this technique maintained viability over 14 days in culture.

## CONCLUSIONS

Tendon injuries give rise to significant morbidity, and at present only limited scientifically proven management modalities exist. A better understanding of tendon pathology, function and healing will allow specific treatment strategies to be developed. Several interesting techniques are being pioneered. We acknowledge that in this field it is likely that several studies with negative findings will never be published. As a result, largely positive findings are only described, and this could be misleading, and even potentially incorrect. The optimization strategies discussed in this article are currently at an early stage of development. Whilst these emerging technologies may develop into substantial clinical treatment options, their full impact needs to be critically evaluated in a scientific fashion.

# Chapter 3

## Tendon augmentation grafts

## ABSTRACT

**Introduction:** Several biomaterials are available to bridge large tendon defects or reinforce tenuous tendon repairs.

**Methods:** We performed a comprehensive search of PubMed, Medline, Cochrane, CINAHL, and Embase databases using various combinations of the commercial names of each scaffold and the keywords "tendon", "rotator cuff", "supraspinatus tendon", "Achilles tendon", "scaffold", "biomaterials", "extracellular matrix", "substitute", and "devices" over the years 1966–2009. All articles relevant to the subject were retrieved, and their bibliographies hand searched for further references in the context to biomaterials for tendon repair.

**Results:** Many biomaterials are available for tendon augmentation. Scanty evidence is available for the use of these scaffolds.

**Discussion:** The emerging field of tissue engineering holds the promise to use biomaterials for tendon augmentation. Preliminary studies support the idea that these biomaterials have the ability to provide an alternative for tendon augmentation. However, available data are lacking to allow definitive conclusion on the use of biomaterials for tendon augmentation. Additionally, the prevalence of postoperative complications encountered with their use varies within the different studies.

**Conclusion:** Rather than providing strong evidence for or against the use of these materials for tendon augmentation, this study instead generates potential areas for additional prospective investigation.

## INTRODUCTION

Tendon disorders are frequent, and are responsible for much morbidity both in sport and the workplace. Incomplete healing of tendon injuries can lead to marked dysfunction and disability, with compromised joint biomechanics and debilitating pain. Clinical approaches to tendons rupture often involve surgical repair, which frequently implies working with degenerative, frayed tendon tissue, unable to sustain the rigors of normal activities, and may fail again. Management of large tendon defects can present a dilemma to the orthopaedic surgeon. Tendon augmentation can provide a more effective management option producing a stronger construct. Surgeons may tackle these injuries using autografts, allografts, xenografts and tendon prosthesis [40]. Allografts and xenografts have become increasingly popular for tendon and ligament repair to overcome the limited availability and donor site complication encountered with the use of autograft tissue [114].

In the last few decades, biomaterials have become critical components in the development of effective new medical therapies for wound care [40, 133]. Many new tissue engineered materials have been introduced: artificial polymers, biodegradable films and biomaterials derived from animals or human, using a combination of principles of engineering and biology [40]. As limitations of previous generations of biologically derived materials are overcome, many new and impressive applications for biomaterials are being examined.

Biological scaffolds are protein-based extracellular matrices which usually derive from human or animal connective tissues [114]. Advantages of biological scaffolds are a well-defined 3D surface proteins microstructure (allowing host cell integration), and natural porosity (which provide much larger space for host cell attachment, proliferation, migration and assists gas and metabolite diffusion). These proprieties allow biological scaffolds to quickly interact with host tissue and induce new tissue formation faster than synthetic scaffolds. Limitations of biological scaffolds are low mechanical properties (often resulting in failure of surgery), nonspecific induction ability, undefined degradation rate, variation in biocompatibility depending on the source of raw materials, which can cause inflammatory response and even implant rejection [114].

On the other hand, synthetic scaffolds are manufactured from chemical compounds [114], which permit better control of the chemical and physical properties leading to stronger mechanical strength and consistency in quality. However, biocompatibility of synthetic scaffolds is very poor, as they can never be absorbed or integrated into host tissue. High incidences of postoperative infection, and chronic immune response have been reported with the use of such materials [114].

The most popular commercially available scaffolds are GraftJacket® (Wright Medical, TN, USA), TissueMend® (Stryker Orthopedics, NJ, USA), Restore™ (DePuy Orthopedics, IN, USA), CuffPatch® (Arthrotek, IN, USA), Zimmer patch formerly known as Permacol™ (Zimmer, IN, USA), Shelhigh No-React® Encuff Patch (Shelhigh Inc., NJ, USA), OrthADAPT®

(Pegasus Biologic Inc., CA, USA), Gore-Tex® patch WL (Gore and Associates, Flagstaff, AZ, USA), Bio-Blanket® (Kensey Nash Corp., PA, USA), Lars® ligament (Dijon, France), Leeds–Keio® or Poly-tape® (Xiros plc, Neoligaments, Leeds, UK; Yufu Itonaga Co., Ltd Tokyo Japan) and Artelon® & Sportmesh™ (Artimplant AB, Sweden & Biomet Sports Medicine, IN, USA) [114] (Table 1). Porcine renal capsule matrix has also been evaluated as a device to repair Achilles tendon injury, resulting equivalent to SIS and meriting further study in other tendon injury models [616].

While the animal-derived products have been FDA 510(k)–approved for reinforcement of soft tissues, human-derived ECM grafts are classified as human tissue for transplantation under the Code of Federal Regulations (21 CFR, part 1270) and they do not require FDA approval for use [40].

Rotator cuff and Achilles tendon injuries repair using these materials have been sparsely documented in the literature. The aim of this paper is to review the current state of knowledge in the field of biomaterials for augmentation of rotator cuff and Achilles tendon injuries.

**Table 1:** The most popular commercially available scaffolds

| Product | Company | Source | Cross-linking | Regulatory approval |
|---|---|---|---|---|
| **Artelon® & Sportmesh™** | (Artimplant AB, Sweden & Biomet Sports Medicine, IN, USA) | Polyurethane urea polymer | Not applicable | Canada, Europe, FDA Artimplant AB, Sweden |
| **Bio-Blanket®** | (Kensey Nash Corp., PA, USA) | Bovine dermis | Yes | FDA |
| **CuffPatch®** | (Arthrotek, IN, USA) | Porcine small intestinal submucosa (SIS) | Yes | FDA |
| **Gore-Tex® patch WL** | (Gore and Associates, Flagstaff, AZ, USA) | Polytetrafluoroethylene | Not applicable | FDA |
| **GraftJacket®** | (Wright Medical, TN, USA) | Human cadaver dermis | No | FDA |
| **Lars® ligament** | (Dijon, France) | Terephthalic polyethylene polyester | Not applicable | Canada, Europe |
| **Leeds–Keio® or Poly-tape®** | (Xiros plc, Neoligaments, Leeds, UK; Yufu Itonaga Co., Ltd Tokyo Japan) | Polyester ethylene terephthalate | Not applicable | Canada, Europe, FDA |
| **OrthADAPT®** | (Pegasus Biologic Inc., CA, USA) | Equine pericardium | Yes | FDA |
| **Permacol™** | (Zimmer, IN, USA) | Porcine dermis | Yes | FDA |
| **Restore™** | (DePuy Orthopedics, IN, USA) | Porcine small intestine submucosa | No | US FDA |
| **Shelhigh No-React® Encuff Patch** | (Shelhigh Inc., NJ, USA) | Bovine or porcine pericardium | Yes | FDA |
| **TissueMend®** | (Stryker Orthopedics, NJ, USA) | Fetal bovine dermis | Yes | FDA |

## METHODS

### Literature search and data extraction

We performed a comprehensive search of PubMed, Medline, Cochrane, CINAHL, and Embase databases using various combinations of the commercial names of each scaffold and the keywords “tendon”, “rotator cuff”, “supraspinatus tendon”, “Achilles tendon”, “scaffold”, “biomaterials”, “extracellular matrix”, “substitute”, and “devices” over the years 1966–2009. All articles relevant to the subject were retrieved, and their bibliographies hand searched for further references in the context to biomaterials for tendon repair. Given the linguistic capabilities of the research team, we considered publications in English, Italian, French, Spanish and Portuguese. The search was limited to articles published in peer-reviewed journals. We excluded from our investigation case reports, literature reviews, and letter to editors. Article reporting on scaffolds for ligament repair were also excluded from the study.

### Commercially available biomaterials

*Biological scaffolds*

Biological scaffolds are obtained from mammalian (human, porcine, bovine and equine) tissues [114]. To remove any non-collagen components, thus minimising the risk of host rejection while retaining its natural collagen structure and mechanical properties, small intestine submucosa, dermis, and pericardium are processed through cascade steps, including general cleaning, removal of lipids or fat deposits, disruption of cellular and DNA materials, cross-linking, and sterilization [114].

The final scaffolds are composed mainly of naturally occurring collagen fibres, predominantly type I collagen, and several of them have a surface chemistry and native structure that is bioactive and promotes cellular proliferation and tissue ingrowth [114].

Restore, GraftJacket, Zimmer, TissueMend, CuffPatch, Shelhigh No-React Encuff Patch, OrthADAPT and Bio-Blanket are considered biological scaffolds [114].

### Small Intestinal Submucosa Xenografts

*CuffPatch*

CuffPatch (Organogenesis, Canton, MA, licensed to Arthrotek, Warsaw, IN) is obtained from porcine small intestine submucosa (SIS). It is composed of 97% collagen and 2% elastin. It has eight layers, it is acellular, and it is provided in a 6.5 by 9 cm sheet [53]. To ensure collagen content maturity, SIS is harvested from a closed herd in pigs weighing at least 205 kg.

The raw material is mechanically processed through a series of customized rollers and the inner and outer mucosal and muscular layers are removed to determine an uniform base product. The machined tissue is then cut and processed with a series of chemical cleansing solutions.

A nondetergent, nonenzymatic chemical cleaning protocol removes cells and cellular debris from SIS and protects the tissue architecture by controlling swelling of the collagen fibers [2]. Following lamination of the individual small intestine submucosa layers, eight layers of the purified material are aligned along the long axis of the intestine and stacked on top of each other. The product is cross-linked with water-soluble carbodiimide. CuffPatch is nominally 0.6 mm thick, and although it is packaged hydrated, it should be rinsed before using.

**Restore graft**

The Restore graft (Depuy, Warsaw, IN) is a circular implant consisting of 10 not cross-linked layers of porcine SIS, 0.8 to 1 mm thick and with a 63 mm diameter. It is more than 90% collagen with approximately 5% to 10% lipids and a small amount of carbohydrate [53, 133]. The layers are obtained from specific pathogen free swine. The inner mucosa and muscular layers are manually removed. Individual SIS sheets are then cleansed and disinfected with peracetic acid and ethanol, and do not contain viable cells.

Ten individual layers are oriented at approximately 20° relative to each other and laminated together under a vacuum press to produce a 1 mm thick isotropic graft with sufficient strength and mechanical properties. Electron beam sterilization is performed after packaging.

Each lot is tested for bacterial endotoxins and mechanical strength. The implant is packaged dry and requires soaking for 5 to 10 minutes before use.

**Dermal Allograft**

*Graftjacket*

Graftjacket (Wright Medical Technology, Inc., Arlington, TN) is a commercially available acellular dermal matrix obtained from tissue bank human skin. It is in compliance with the American Association of Tissue Banks guidelines for allograft material, and it is classified as human tissue for transplantation.

The skin is processed with a patented technique which removed epidermal and dermal cells, and the Graftjacket is then freeze-dried to prevent the formation of ice crystals and to retain the native extracellular architecture and vascular channels.

Because it is rendered acellular during processing, it lacks many of the disadvantages typical of standard allograft tissue. The resulting patch is an acellular tissue composed of collagen types I, III, IV, VII, elastin, chondroitin sulfate, proteoglycans, and fibroblast

growth factor. It has an intact basement membrane complex and preserved vascular channels to allow rapid infiltration of fibroblasts and vascular tissue, with minimal host inflammatory response [6, 133, 162].

It is recommended for tendon repairs, ligament augmentation, capsular reinforcement, and periosteal covering [133]. It is commercially available in several forms. With an average thickness of 1.0 mm, it is available in 5 by 5 and 5 by 10 cm sheets. With an average thickness of 1.5 mm, it is available in 4 by 7 or 5 by 5 cm sizes. With an average thickness of 2.0 mm, it is available in a 4 by 7 mm size. It is packaged dry. Before use, the Graftjacket needs to be hydrated for at least 10 to 15 minutes [133].

*AlloPatch*

Allopatch HD is derived from human allograft skin processed using proprietary procedures developed by the Musculoskeletal Transplant Foundation. It is commercially available in several forms. With an average thickness of 0.8 mm – 1.7 mm, it is available in 5 by 5, 2 by 5, and 4 by 8 cm sheets. With an average of thickness ≥ 1.8 mm, it is available in 4 by 8 or 5 by 5 cm sizes. With an average thickness of 0.4 mm – 0.7 mm, it is available in a 2 by 5 cm size.

## Dermal Xenografts

*The Zimmer Collagen Repair patch*

The Zimmer Collagen Repair patch (Tissue Science Laboratories, Covington, GA, licensed to Zimmer, Warsaw, IN), is a single layer porcine skin xenograft. It is an acellular cross-linked collagen sheet of cross-linked porcine dermis, 1.5 mm thick on average. After the initial mechanical processing to remove hair and epidermis, acetone is used to saponify the graft. Organic and enzymatic extractions are undertaken to remove fat, cellular material, and soluble proteins. Hexamethylenediisocyanate cross-linking is then performed. The Zimmer Collagen Repair patch may be stored at room temperature and is packaged hydrated

*TissueMend*

TissueMend (TEI Biosciences, Boston, MA, licensed to Stryker Howmedica Osteonics, Kalamazoo, MI) is a single layer acellular, nondenatured collagen membrane derived from fetal bovine dermis, nominally 1 mm thick. The material is aseptically processed to remove cells, lipids, and carbohydrates to reduce antigenicity and cleanse the tissue, and then sterilized in ethylene oxide. The product is 99% nondenatured fetal bovine collagen, which is not artificially cross-linked. It is available as a rectangular 5 by 6 cm implant and was tested in 2 thicknesses: 1.1 mm and 1.2 mm. It is lyophilized and packaged dry. The hydratation process requires less than 1 minute.

*BioBlanket*

BioBlanket (Kensey Nash Corporation) Surgical Mesh is a porous tissue bovine dermal tissue matrix composed of a proprietary blend of fibrous and acid soluble collagens. It is lyophilized, crafted and cross-linked with proprietary processing methods to maintain mechanical and degradation profiles while the native tissue heals. Finally, the mesh is sterilized by irradiation. It has been FDA approved for the reinforcement and repair of a variety of soft tissues.

**Pericardial Xenograft**

*OrthoADAPT*

OrthoADAPT (Pegasus Biologics, Irvine, CA) is an acellular biologic scaffold derived from equine pericardium. It is cross-linked and sterilized with a proprietary process of biodegradable agents. It is not irradiated. It is approximately 90% type I collagen and 10% type II collagen. It is the thinnest graft available at 0.5mm and is available as a 3 by 3 or 4 by 5 cm sheet or in strips that can be integrated into repairs.

**Fascia lata**

*AlloPatch*

AlloPatch human fascia lata (Musculoskeletal Transplant Foundation) provides high peak load and tensile strength. A proprietary accellularization process leaves the human collagen matrix intact. Freeze-dried and packaged flat, Allopatch rehydrates in minutes, and stores at room temperature.

*Synthetic scaffolds*

Synthetic scaffolds are made of polyester, polypropylene, polyarylamide, dacron, carbon, silicone and nylon [114]. They have superior mechanical characteristics compared with biological scaffolds, but very poor biocompatibility, and may cause several long-term complications [114].

*Shelhigh No-React® Encuff Patch*

Shelhigh No-React® Encuff Patch (Shelhigh Inc., NJ, USA), is a subcategory of Shelhigh No-React patch, which was previously used in abdominal surgery [501]. The brand name is better known for its artificial vascular valve products, which have been detoxified through a proprietary No-React process that makes the scaffold more resistant to adhesion degradation, dilation, infection and calcification [114].

*Lars® ligament*

The Lars® ligament (Dijon, France) is a second-generation, nonabsorbable synthetic ligament device made of terephthalic polyethylene polyester fibers [274]. It has been approved by the health authorities of Canada, Europe and several other countries, but not the USA, for a range of applications [114].

*Leeds–Keio® or Poly-tape®*

The Leeds–Keio® or Poly-tape® (Xiros plc, Neoligaments, Leeds, UK; Yufu Itonaga Co., Ltd Tokyo Japan) is made of polyester (ethylene terephthalate) and was developed by the University of Leeds and the Keio University hence its name [114]. The Leeds–Keio was specifically designed for ACL reconstruction with stiffness of 200 N/mm, similar to that of natural ACL [421].

*Artelon® & Sportmesh™*

The Artelon® and Sportmesh™ (Artimplant AB, Sweden & Biomet Sports Medicine, IN, USA) Artelon (Artimplant AB, Sweden) and Sportmesh (Biomet Sports Medicine, IN, USA) are made of biodegradable polyurethane urea polymer. It has been cleared by the CE and FDA for reinforcement of soft tissues, including rotator cuff, Achilles, patellar, biceps, quadriceps [114]. The device is supplied sterile in sheet form in double layer peelable packaging.

*Gore-Tex® patch WL*

The Gore-Tex® patch WL (Gore and Associates, Flagstaff, AZ, USA) is composed of the inert biomaterial expanded polytetrafluoroethylene (ePTFE). It features a microporous structure allowing for host tissue incorporation [261]. It is elastic and resembles a dense sponge rubber [327]. The manufacturers have reported an *in vitro* study on the strength of a 2 mm thick Gore-Tex soft tissue patch, as well as on that of Marlex Mesh, Prolene Mesh, and Mersilene Mesh patches. The maximum force at rupture was 11.0 kg/cm, 4.1 kg/cm, 6.4 kg/cm, and 2.3 kg/cm, respectively.

## ROTATOR CUFF

### Laboratory studies

Laboratory studies on biomaterials and rotator cuff are reported in table 2.

**Table 2:** Preclinical Studies on Rotator Cuff

| **Preclincal Studies on Rotator Cuff** | | | |
|---|---|---|---|
| **Author** | **Product** | **Model** | **Tendon** |
| **Dejardin [156], 2001** | | Dog | Infraspinatus |
| **Zheng [697], 2005** | Restore | Rabbit | Supraspinatus |
| **Zalavras [690], 2006** | | Rat | Supraspinatus |
| **Schlegel [565], 2006** | | Sheep | Infraspinatus |
| **Perry [507], 2007** | Restore | Rat | Rotator Cuff |
| **Chen [115], 2007** | Restore | Rabbit | Rotator Cuff |
| **Adams [6], 2006** | GraftJacket | Dog | Infraspinatus |
| **Sano [557], 2002** | | Rabbit | Supraspinatus |
| **Nicholson [464], 2007** | Zimmer Collagen Patch | Ewe | Infraspinatus |
| **Cole [126], 2006** | Polycarbonate Polyuretane Patch | Rat | Supraspinatus |
| **Koh [317], 2002** | Polylactic acid | Sheep | Infraspinatus |
| **Mac Gillivray [387], 2006** | Polylactic Acid | Goat | Infraspinatus |
| **Moffat [444], 2009** | PLGA-nanofiber based scaffold | Laboratory study | Rotator cuff |

*1. Porcine SIS*

Dejardin et al [156] used porcine SIS in a canine infraspinatus injury model. Gross appearance, histological continuity, and failure mode of the device evaluated at 3 and 6 months were similar to native tendon with a good integration between the new tendon and bone.

Zheng et al [697] used Restore SIS in a rabbit supraspinatus injury model. Histologic evaluation at 8 weeks showed total replacement by collagen fibers in 4 of 5 samples, and no significant differences with the autologus implant, but the overall histology scores achieved by SIS implantation were still poorer than that of the autologous tendon implant.

Zalavras et al [690] used a SIS device as an interpositional graft in a rat supraspinatus injury model. Histology and biomechanical testing at 6 and 16 weeks showed neovascularization and fibroblastic ingrowth in SIS-regenerated tendons, with an ultimate force to failure 78% of normal at 16 weeks. This was higher than in the defect group, which demonstrated an ultimate force to failure 34% of normal. The ultimate force to failure of the SIS-regenerated tendons approached that of the normal tendon at 16 weeks.

Schlegel et al [565] used an SIS device to augment infraspinatus tendon repair in an ovine shoulder model. At 12 weeks, biomechanical testing and histology were performed. Histology addressed tissue healing at the bone-tendon interface. Although none of the patches were intact, the load-to-failure data did not indicate a significant difference between the augmented and non-augmented groups. However, the augmented group had significantly better stiffness than the non-augmented group. Histology showed that the infraspinatus tendon in all specimens inserted into the bone through a zone of fibrocartilage, although none of the patches were intact.

Perry et al [507] used the Restore device in rat models of acute and chronic rotator cuff tear. Geometric measures and mechanical testing showed similar properties between the acute injury model and the injury repaired without SIS, while the chronic repair injury model showed an increased modulus and a lower cross sectional area of the healing tendon.

Chen et al [115] used Restore and type I/III collagen bioscaffold as bioscaffold carriers for autologous tenocytes in a rabbit model of massive rotator cuff defect. At 8 weeks, the inflammatory reactions of both tenocyte-seeded bioscaffolds were dramatically less than with bioscaffold alone. In addition, bioscaffolds seeded with tenocytes produced a histological appearance similar to that of the positive control.

*2. GraftJacket*

Adams et al [6] investigated the use of GraftJacket as an interpositional graft in a canine infraspinatus tendon injury model. Histologically, by 6 weeks cells infiltrated the control and experimental specimens. Biomechanically, by 12 weeks the strength of the experimental repair was equal to that of the control, but lower than that of the normal tendon. At 6 months, control and experimental specimens mimicked normal tendon structure grossly and histologically.

*3. Fresh autograft fascia lata*

Sano et al [557] investigated the use of fresh autograft fascia lata as an interpositional graft in a rabbit supraspinatus injury model. At the fascia-bone junction, chondrocytes started to appear at 2 weeks after surgery, and increased rapidly thereafter in number and columnar organization. By 8 weeks, remodeling of direct insertion with fibrocartilage was almost complete, although a tidemark was not observed. The distribution of collagen types II and III showed a pattern similar to that of a normal supraspinatus tendon-bone insertion. The biomechanical properties were not reported.

*4. Zimmer Collagen Patch*

Nicholson et al [464] evaluated Zimmer Collagen Repair (porcine dermal, PD) patch and Restore (SIS) patch in an *in vivo* sheep infraspinatus injury model. Bilateral infraspinatus tears were created and repaired in 2 groups of 8 adult ewes. Each group (killed at 9 or 24 weeks) included 5 repaired with suture alone, 6 repaired and augmented with a (PD) patch, and 5 repaired and augmented with a SIS patch. At 9 weeks, the suture-only repair exhibited normal connective tissue formation. The PD patches were intact but were not fully integrated with surrounding tendon tissues at this time point. A large number of giant cells on the PD surface plus fibroblasts, macrophages, and lymphocytes were seen. There was no connective tissue interdigitation at 9 weeks. The majority of SIS patches appeared to be completely resorbed. The area of the resorbed SIS patches was surrounded by primitive connective tissue containing macrophages, fibroblasts, woven bone, and new

cartilage. At 24 weeks, failure loads were the same between groups, macrophages had disappeared from the PD groups, and integration of the PD patch into the surrounding tissue with vascular and fibroblastic invasion was seen.

*5. Polycarbonate polyurethane*

Cole et al [126] investigated the biological response to a novel polycarbonate polyurethane patch used for tissue augmentation in a rat supraspinatus injury model. By 6 weeks, histology demonstrated no inflammatory reaction, and histomorphometry showed an average patch infiltration with connective tissue of 79.9%.

*6. Polylactic acid*

Koh et al [317] augmented a sheep infraspinatus tendon repair with a polylactic acid scaffold. The augmented repair demonstrated a 25% greater strength than the non-augmented repair.

MacGillivray et al [387] used polylactic acid patch to repair a goat infraspinatus defect model. There was no significant difference in load to failure between the shoulders repaired and augmented with polylactic acid patch and those repaired but not augmented. At 6 weeks, a cellular fibrous tissue occupied the patch, then maturing into a dense and homogeneous fibrous tissue with alignment of collagen between the scaffold bundles.

*7. PLGA nanofiber-based scaffold.*

Moffat et al [444] designed a poly (lactide-co-glycolide) (PLGA) nanofiber-based scaffold for rotator cuff tendon tissue engineering. Rotator cuff fibroblasts cultured on the aligned scaffolds attached along the nanofiber long axis, while the cells on the unaligned scaffold were polygonal and randomly oriented. Quantitative analysis revealed that cell alignment, distribution, and matrix deposition conformed to nanofiber organization and that the observed differences were maintained over time. Mechanical properties of the aligned nanofiber scaffolds were significantly higher than those of the unaligned ones, and, although the scaffolds degraded *in vitro*, physiologically relevant mechanical properties were maintained, demonstrating the potential of the PLGA nanofiber-based scaffold system for functional rotator cuff repair. Moreover, nanofiber organization has a profound effect on cellular response and matrix properties, a critical parameter for scaffold design.

**Comparison studies**

Derwin et al [162] compared the properties of GraftJacket TissueMend Restore and CuffPatch and their elastic moduli with that of normal infraspinatus canine tendon. Restore and CuffPatch had higher moduli than GraftJacket and TissueMend but that the elastic moduli of commercial extracellular matrices were one order of magnitude lower than that of canine infraspinatus tendons. The extracellular matrix moduli were one order of magnitude

lower than the moduli (grip-to-grip strain) reported for different regions of the human infraspinatus tendon, suggesting that these extracellular matrices would likely carry only small loads.

## Clinical studies

Clinical studies on biomaterials and rotator cuff are reported in table 3.

**Table 3:** Clinical Studies on Rotator Cuff

| **Clinical Studies on Rotator Cuff** | | | | |
|---|---|---|---|---|
| **Author** | **Product** | **Tendon** | **Number of patients** | **Failure** |
| **Metcalf [432], 2002** | Restore | Rotator Cuff | 24 | 1 |
| **Sclamberg [575], 2004** | Restore | Rotator Cuff | 11 | 10 |
| **Zheng [697], 2005** | Restore | Rotator Cuff | 4 | 4 |
| **Iannotti [273], 2006** | Restore | Rotator Cuff | 30 | 6/15 control group and 9/15 scaffold group |
| **Walton [660], 2007** | Restore | Rotator Cuff | 24 | 7/12 control group and 6/10 scaffold group |
| **Soler [595], 2007** | Zimmer Collagen Patch | Rotator Cuff | 4 | 4 |
| **Badhe [43], 2008** | Zimmer Collagen Patch | Rotator Cuff | 10 | 2 |
| **Barber [52], 2008** | GraftJacket | Supraspinatus | 17 | 3 |
| **Bond [80], 2008** | GraftJacket | Rotator Cuff | 16 | 3 |

*Porcine SIS/Restore*

Iannotti et al [273] tried to determine the effectiveness of porcine SIS to augment the repair of rotator cuff in humans. They randomised thirty shoulders with a chronic two-tendon rotator cuff tear (nine with a large tear and twenty-one with a massive tear of rotator cuff) that was completely repairable with open surgery to be managed with either augmentation with porcine SIS or no augmentation. The rotator cuff healed in four of the fifteen shoulders in the augmentation group compared with nine of the fifteen in the control group ($p = 0.11$). The authors concluded that augmentation of the surgical repair of large and massive chronic rotator cuff tears with porcine SIS did not improve the rate of tendon-healing or the clinical outcome scores. On the basis of their investigation, they do not recommend using porcine SIS to augment repairs of massive chronic rotator cuff tears performed with the surgical and postoperative procedures described in this study.

Metcalf et al [432] conducted a 2-year follow-up of 12 patients who underwent arthroscopic repair of massive chronic rotator cuff tears using Restore SIS as an augmentation device. Post-operative magnetic resonance imaging (MRI) scans showed significant thickening of the cuff tendon with the incorporation of the SIS graft in 11 patients. In 1 of 12 patients,

clinical failure was observed within 12 weeks with complete resorption of the graft. There was no evidence of local or systemic rejection or infection in any patient. The mean post-operative University of California, Los Angeles (UCLA) score was 19.9 on a scale of 35, a significant improvement over the pre-operative score of 9.9 ($P < .01$), but the shoulder function remained far below normal in these patients. This study demonstrated improved post-operative outcomes for patients managed with Restore graft augmentation compared with their pre-operative condition. However, the lack of a control group makes it difficult to conclude that the functional improvements in the study were the result of SIS augmentation.

Sclamberg et al [575] evaluated clinical and magnetic resonance imaging (MRI) at 6 months in 11 patients undergoing open repair of large or massive rotator cuff tears augmented with Restore. MRI showed a re-tear in 10 of 11 patients.

Zheng et al [697] performed a study to evaluate the safety and efficacy of Restore™ SIS membrane. The Restore™ orthobiologic implant was examined by histology and the nested PCR technique using porcine immunoreceptor DAP12 gene to examine if SIS membrane contained porcine cells or DNA, respectively. The material was also implanted into mice and rabbits for the evaluation of biological reaction and inflammatory response. Restore™ SIS was found to contain multiple layers of porcine cells. Chloroacetate esterase staining showed that some of these cells were mast cells. Nested PCR of the DAP12 gene demonstrated that Restore™ SIS contained porcine DNA material. Subcutaneous implantation of Restore™ SIS membrane in mice, and in rabbits for rotator cuff tendon repair, showed that the membrane caused an inflammatory reaction characterized by massive lymphocyte infiltration. The authors concluded that Restore™ SIS is not an acellular collagenous matrix, and contains porcine DNA, contradicting the current view that Restore ™ SIS is a cell-free biomaterial, and that no inflammatory response is elicited by its implantation.

Walton et al [660] compared a group of patients who had undergone rotator cuff repair with xenograft augmentation with a group repaired without augmentation. Four patients of the xenograft group showed a severe post-operative reaction requiring surgical treatment. Two years post-operatively, MRI documented retears in six of the ten tendons repaired with a xenograft and in seven of the twelve non-augmented tendons; the patients with a xenograft also had less strength than the controls and had more impingement in external rotation, a slower rate of resolution of pain during activities, more difficulty with hand-behind-the-back activities, and a lower rate sports participation.

*1. Zimmer Collagen Patch*

Soler et al [595] used Zimmer Collagen Patch as a bridging device to repair massive rotator cuff tears. After a good post-operative period, between 3 and 6 months the graft began to fail and the patients showed signs and symptoms of retear, with also signs of inflammation. MRI scans showed inflammatory changes, resorption of the graft, fluid pooling in

the subdeltoid bursa and loss of continuity of the remaining graft material. Histology of the debris revealed necrotic fibrinous material on a background of chronic inflammation.

Badhe et al [43] prospectively evaluated 10 patients with extensive rotator cuff tear treated with Zimmer Collagen Patch (Permacol). All patients experienced significant pain relief, and improvement in abduction power and range of motion. Ultrasound imaging at the final follow up identified intact grafts in 8 and disrupted grafts in 2 patients.

*2. GraftJacket*

Barber et al [52] compared the failure mode of supraspinatus tendon repair with and without Graftjacket augmentation in a human cadaveric model. No significant displacement occurred during the cyclic phase, and no anchors failed. During the destructive testing phase, the mean load-to-failure strength of the control construct was 273 +/− 116 N. The load-to-failure strength of the supraspinatus tendon augmented with GraftJacket was 325+/− 74 N. The constructs failed by 2 different mechanisms: tendon-suture interface failure (8/10 non-augmented repairs and 6/10 augmented repairs) and suture breakage (2/10 non-augmented repairs and 4/10 augmented repairs).

Bond et al [80] treated 16 patients with massive rotator cuff tears with arthroscopic implantation of a GraftJacket allograft. At mean follow-up of 26.7 months, 15 of 16 patients were satisfied with the procedure. The mean UCLA score increased from 18.4 pre-operatively to 30.4 post-operatively. The mean pain score improved from 4.6 to 9.8 post-operatively. The mean Constant score increased from 53.8 to 84.0. Statistically significant improvements were noted in pain, forward flexion and external rotation strength. MRI scans showed full incorporation of the graft into the native tissue in 13 patients.

## Achilles tendon

*Laboratory studies*

Laboratory studies on biomaterials and Achilles tendon are reported in table 4.

**Table 4:** Preclinical Studies on Achilles Tendon

| **Preclinical Studies on Achilles Tendon** | | |
|---|---|---|
| **Author** | **Type** | **Model** |
| **Foster [192], 1978** | Polymer filamentous carbon composites | Rabbit |
| **Alexander [12], 1983** | Polymer filamentous carbon composites | Rabbit |
| **Bonutti [81], 1988** | Isobutyl Cyanoacrilate | Rabbit |
| **Badylak [44], 1995** | SIS | Dog |
| **Zantop [692], 2006** | SIS | Mouse |
| **Gilbert [226], 2007** | SIS | Dog |
| **Suckow [616], 2007** | Renal Capsule | Rat |

*1. Polymer filamentous carbon composites*

Foster et al[192] used filamentous carbon fiber to replace the Achilles tendon in a rabbit model: carbon-induced "neotendon" rapidly developed from young fibroblastic tissue outgrowths of the loose mesenchymal tissue of the perineurium and adventitia of the blood vessels in the adjacent neurovascular bundle.

Alexander et al[12] used a composite material of filamentous carbon coated with an absorbable polymer, polylactic acid (PLA), as a tissue scaffold in rabbit Achilles tendons. The resumption of activity was possible with good histological and mechanical outcomes.

*2. Isobutyl cyanoacrilate*

Bonutti et al[81] used isobutyl cyanoacrilate (ICA) in a rabbit Achilles tendon injury model: ICA alone exhibits reasonable strength *in vitro.* In combination with suture, ICA provides a stronger initial repair than either suture or adhesive alone.

*3. SIS*

Badylak et al[44] used SIS in a dog model of Achilles tendon defect. By 12 weeks post-operatively SIS remodeled neotendons were stronger than the musculotendinous origin or the bony insertion (>1000N), and showed organized collagen-rich connective tissue similar to the normal tendons. The dogs in which no SIS was implanted showed inferior strength. Immunohistochemical studies showed SIS degradation within the first eight weeks, demonstrating that it behaves as a temporary scaffold for the organization of connective tissue.

Zantop et al[692] demonstrated that bone marrow-derived cells participate in the long-term remodelling of the Achilles tendon in a mouse model repaired with a SIS-ECM scaffold. The device recruited a population of bone marrow-derived cells that participated in the long-term remodelling process.

Gilbert et al[226] analyzed the temporal degradation of the SIS device used for the repair of Achilles tendon in a dog model. There was a rapid degradation, with approximately 60% of the mass lost by one month after surgery, and complete resorption of the graft by three months. Histology at 3 months showed that the graft supported rapid cellular infiltration and host tissue ingrowth, with a dense collagenous tissue with organization, cellularity, and vascularity similar to that of normal tendon.

*4. Porcine renal capsule*

Suckow et al[616] studied the utility of porcine renal capsule matrix (RCM) in comparison to SIS in a rat Achilles tenotomy repair model. Rats treated with RCM had slightly higher scores for degree of histologic change, suggesting a more rapid repair of the tenotomy site than in SIS-treated. While remnants of SIS surrounded by macrophages and multi-

nucleated giant cells were still present in some rats, remnants of RCM were not observed, suggesting more rapid incorporation of RCM.

*5. Bone Marrow Stromal Cell-Seeded Knitted PLGA Fiber Scaffold*

Ouyang et al evaluated the effect of marrow-stromal-cell (bMSC)-seeded knitted PLGA scaffold for Achilles tendon repair in two studies on rabbit models. In the first study [480], both the groups of tendons repaired with knitted PLGA graft (seeded with bMSC or not) showed good attachment of the scaffold to the proximal and distal ends of tendon 2 weeks post-operatively, but the volume of regenerated tissue was greater in the bMSC–seeded group. Immunoistochemistry showed that the cells were able to synthesize collagen. Histology showed more eosinphilic tissue formation inside and around the scaffold and more mature collagen fibers in bMSC/PLGA treated tendons than in the others. Perhaps PLGA scaffolds allowed cell infiltration, tissue formation, and were absorbed gradually after the formation of neotissue by the host. In the second study [481], at 2 and 4 weeks the histology of the specimens bMSC/PLGA treated exhibited a higher rate of tissue formation and remodelling compared with specimens treated with PLGA alone, whereas at 8 and 12 weeks after the procedure. The histology of both groups was similar to that of native tendon tissue. The wound sites of group bMSC/PLGA treated healed well, and showed no apparent lymphocyte infiltration. The tensile stiffness and modulus of group bMSC/PLGA treated were greater than those of the group treated with PLGA only.

## CLINICAL STUDIES

Clinical studies on biomaterials and Achilles tendon are reported in table 5.

**Table 5:** Clinical Studies on Achilles Tendon

| **Clinical Studies on Achilles Tendon** | | | |
|---|---|---|---|
| **Author** | **Product** | **Number of patients** | **Failure** |
| **Parsons [496], 1989** | Polymer filamentous Carbon Compsites | 48 | No increased morbidity with the use of the carbon implant |
| **Lee MS [336], 2004** | GraftJacket | 1 | None |
| **Lee DK [333], 2007** | GraftJacket | 9 | None |
| **Lee DK [334], 2008** | GraftJacket | 11 | None |

*1. Polymer filamentous carbon composites*

Parsons et al [496] used an implant composed of filamentous uniaxially aligned carbon fibers coated with an absorbable polymer in 48 patients with a rupture of Achilles tendon. This

device acted as a scaffold for regrowth of collagenous tissue. The early strength of this repair was provided by the composite implant and by the rapid ingrowth and attachment of new tissue. All patients demonstrated continuous improvement during the first post-operative year, and a high level of function throughout the second year. Both repair of chronic and acute injury greatly improved.

*2. GraftJacket*

Lee [336] described the augmentation of chronic Achilles tendon rupture repair with Graft-Jacket, noting early return to activity and good plantarflexion strength.

Lee conducted two studies to evaluate Graftjacket as an augmentation device in Achilles tendon repair. In the first study [333], nine patients with chronic Achilles tendon ruptures were followed up. There were no re-ruptures or recurrent pain at 20 to 30 months post-operatively, and the average return-to-activity time was 15.2 +/− 1.7 weeks.

In the second study [334], 11 patients with acute tendon ruptures were followed up for 20 to 31 months. At 20-months, there were no re-ruptures or recurrent pain; the average return-to-activity time was 11.8 +/− 0.75 weeks.

Barber et al [55] demonstrated a significant increase in strength and stiffness of Achilles tendon repair augmented with GraftJacket in a human cadaver model (12.99+/−5.34 N/mm vs 4.29+/− 0.83 N/mm of the control group).

**Comparisons of different graft materials**

Kummer et al [327] examined four different graft materials (Gore-Tex Soft Tissue Patch, Graftjacket, bovine pericardium, and an experimental graft material from Xylos Corporation) in chicken Achilles tendons. Compared to non-augmented suture, grafts increased suture fixation strength from 10% to 60% in shear and from 0% to 36% in pull-off with the bovine pericardium graft, providing significant improvement in both tests. In no cases (even unaugmented) did the suture pull directly through the tendon, but sliced along it, demonstrating that the interface between the suture and the tendon determines fixation strength. Grafts function by increasing the area, friction, and nature of this interface, not by acting as a barrier for suture pull-through.

## DISCUSSION

The emerging field of tissue engineering holds the promise to use materials in tendon injury repair, namely artificial polymers, biodegradable films and biomaterials derived from animals or human (ECM devices) [53]. The most innovative strategy in tendon injury repair is the use of ECM matrices. In contrast to traditional polymeric and metallic orthopaedic devices,

intended to restore mechanical function and remain unchanged for the life of the patient, ECMs are temporary scaffold aimed to enhance and accelerate the biology of tissue repair [162]. They undergo host cell infiltration and constructive tissue remodeling at variable rates [525].

Potential advantages of the use of ECM grafts include the capability to decrease the *in vivo* mechanical forces on the tendon repair during post-operative healing, to prevent repair gap formation or failure, to allow host cell infiltration and ideally even enhance the biology healing, and to be replaced by organized host tissue over time. Additional research studies are required to verify these issues.

The ideal scaffold should induce host tissue ingrowth and tendon regeneration during the process of degradation, which varies dramatically among the commercially available scaffolds [647]. The capability of inducing host tissue ingrowth is superior when using biological scaffolds, even though this process appears uncontrolled and nonspecific [239].

The interaction between scaffold surface and host cells is a key aspect of the use of scaffolds for tendon reconstruction. In the first phase of cellular ingrowth, multiple attachment points are established by the cells through the interaction between transmembrane proteins and proteins at the scaffold surface [114], later strengthened by accumulating integrin receptors, eventually forming a focal adhesion which acts as a connection between the actin cytoskeleton of the cell and the surface [114]. The cell proliferation cycle and cell migration start after the formation of focal adhesions and spreading of cells on the surface [114]. Cell attachment, proliferation and migration is facilitated by the porosity of scaffolds [272].

The surface of biological scaffolds is mostly composed of natural type I collagen protein, which determines a higher affinity to host cells and therefore promotes cellular adhesion, proliferation, migration and tissue induction [114]. On the other hand, the surfaces of synthetic scaffold are composed of macromolecules lacking a well-defined structure that allows host cell to produce a strong binding point and start growing [114].

Even though biologic scaffolds are becoming more popular, clinical well conducted human studies are lacking, and little data describing the complications or adverse events associated with the use of these products are available. ECMs fabricating in parallel with other materials may increase their mechanical properties, such as natural ECMs seeded with bone marrow stem cells or tenocytes [480, 481]. However, clinical evidences in this field are scanty.

Major concern about both biological and synthetic scaffolds is the biocompatibility and the inflammatory response associate with foreign body rejection [114]. To decrease the bio-burden and the risk of inflammatory or foreign body reactions, all tissues, regardless of their origin, are extensively purified to remove proteins, cells, and lipids. Some graft options have been artificially crosslinked to decrease antigenicity, by decreasing their sensitivity to collagenases. Although rare, aseptic, nonspecific inflammatory reactions and foreign body-like reactions have been reported with certain xenografts [53, 64, 133, 413, 697].

Aseptic reactions were reported in 16% to 22% [413] of implantations, always with negative aspirates and cultures, destroyed xenografts, and histopathological evidence of inflamed granulation tissue with abundant neutrophils, but no foreign body reaction, as documented by the absence of organisms, crystals, or giant cells [133, 413].

Valentin et al [647] examined the host-tissue morphologic response to five commercially available extracellular matrix-derived biologic scaffolds (GraftJacket, Restore, CuffPatch, TissueMend, Permacol) used for orthopaedic soft-tissue repair in a rodent model. Each device elicited a distinct morphologic response that differed with respect to cellularity, vascularity, the presence of multinucleated giant cells, and organization of the remodeled tissue. More rapidly degraded devices such as Restore and autologous tissue showed the greatest amount of cellular infiltration, especially at the early time-points. Devices that degraded slowly, such as CuffPatch, TissueMend, and Permacol, were associated with the presence of foreign-body giant cells, chronic inflammation, and/or the accumulation of dense, poorly organized fibrous tissue.

Depending on the product, processing may involve acellularization treatment, chemical cross-linking, lamination of multiple layers, or lyophilization [40]. These biomaterials have incomplete acellularization [162, 697], and the clinical implications are still not clear. Acellularization treatment aims to reduce antigenicity, by disrupting cells and removing water-soluble cellular proteins. Acellularization may also enhance host cell infiltration with phenotypically appropriate cells [301] and possibly prevent transmission of infectious genomic vectors [119]. Further biochemical and immunologic investigations are required to establish whether and how much acellularization treatment increases the safety and efficacy of these implants.

The use of biological scaffolds manufactured from human or animal tissue carries also the risk of disease transmission, which even though not reported to date, remain a theoretical concern. Obviously, there is no risk of disease transmission with the use of synthetic scaffolds [114].

One of the advantages of biomaterials is that exogenous growth factors, gene therapy approaches, or cell delivery can be used together with these biomaterials.

Several chemical cross-linking agents (i.e., glutaraldehyde, polyepoxy compound, carbodiimide, genipin, isocyanate and proanthocyanidin) have been used to stabilize the collagen structure of the scaffold, maintaining the mechanical properties. Clinical studies have not confirmed the expected beneficial effect of chemical cross-linking scaffolds. Further investigations are warranted to establish the *in vivo* benefit of chemical cross-linking in biocompatibility and mechanical properties on the scaffolds [114].

As proposed by Chen et al, another reason of concern is that available scaffolds are produced to mimic the tendon or ligament extracellular microenvironment to stimulate cell proliferation and tissue in-growth, largely ignoring the healing process at the enthesis. The repair procedure often involves reconstruction of the junction and failure of surgery is

frequently caused by osteolysis and scaffold pullout. Further investigations are required to better understand how to promote the healing of bone-tendon junction.

In conclusion, preliminary studies support the idea that these biomaterials can provide an alternative for tendon augmentation with an enormous therapeutic potential. However, available data are lacking to allow definitive conclusion on the use of biomaterials for tendon augmentation. Additionally, the prevalence of postoperative complications encountered with their use varies within the different studies. Rather than providing strong evidence for or against the use of these materials for tendon augmentation, this study instead generates potential areas for additional prospective investigation. Further investigations are required to evaluate the role of these materials in the clinical practice.

# Chapter 4

## Current concepts review: New approaches for the management of tendinopathy

## ABSTRACT

Tendinopathy is a failed healing response of the tendon.

Despite an abundance of therapeutic options, very few randomised prospective, placebo controlled trials exist to assist in choosing the best evidence-based management.

Eccentric exercises have been proposed to promote collagen fibre cross-link formation within the tendon, thereby facilitating tendon remodelling. Overall results suggest a trend for a positive effect of eccentric exercises, with no reported adverse effects. Combining eccentric training and shock wave therapy produces higher success rates compared to eccentric loading alone or shock wave therapy alone.

The use of injectable substances in and around tendons such as platelet-rich plasma, autologous blood, polidocanol, corticosteroids, aprotinin is popular but there is minimal clinical evidence to support their use.

The aim of operative treatment is to excise fibrotic adhesions, remove areas of failed healing and make multiple longitudinal incisions in the tendon to detect intratendinous lesions and to restore vascularity and possibly stimulate the remaining viable cells to initiate cell matrix response and healing.

New operative procedures include endoscopy, electrocoagulation and minimally invasive stripping. These techniques aim to disrupt the abnormal neoinnervation to interfere with the pain sensation caused by tendinopathy.

Randomized controlled trials are necessary to better clarify the best therapeutic options for the management of tendinopathy.

## EVOLVING CONCEPTS IN TENDINOPATHY: NEW THEORIES

Tendinopathies account for a substantial proportion of overuse injuries associated with sports, and are a common cause of disability [21, 259]. Most major tendons, such as the Achilles, patellar, rotator cuff and forearm extensor tendons (amongst others) are vulnerable to overuse, which induces pathological changes in the tendon [530].

The term tendinopathy as a generic descriptor of the clinical conditions (both pain and pathology) associated with overuse in and around tendons [393]. The histological descriptive term 'tendinosis' (a degenerative pathology with a lack of inflammatory change) and 'tendonitis' or 'tendinitis' (implying an inflammatory process) should only be used after histopathological confirmation [393]. However, it should be kept in mind that, despite the use of the term 'tendinosis', at histopathological examination the essence of a tendinopathic lesion is a failed healing response, with haphazard proliferation of tenocytes, intracellular abnormalities in tenocytes, disruption of collagen fibres, and subsequent increase in non-collagenous matrix. Tendinopathic tendons have an increased rate of matrix remodelling, leading to a mechanically less stable tendon which is probably more susceptible to damage [34]. Histological studies of surgical specimens in patients with established tendinopathy consistently show either absent or minimal inflammation. They generally also show hypercellularity, a loss of the tightly bundled collagen fiber appearance, an increase in proteoglycan content and, commonly, neovascularisation. Inflammation seems to play a role only in the initiation, but not propagation and progression, of the disease process [529]. Competing theories have been proposed to explain the pathogenesis of tendon pathology at specific stages and presentations of the condition. A continuum of tendon pathology from asymptomatic tendons to tendon tears has been proposed [132, 341].

Failed healing and tendinopathic features have been associated with chronic overload, but the same histopathologic characteristics also has been described when a tendon is unloaded: stress shielding seems to exert a deleterious effect. Unloading a tendon induces cell and matrix changes similar to those seen in an overloaded state, and decreases the mechanical integrity of the tendon [132, 341].

Despite an abundance of therapeutic options, very few randomised prospective, placebo controlled trials have been conducted to assist in choosing the best evidence-based management. Managements that have been investigated using a randomised controlled trial design include nonsteroidal anti-inflammatory medications [37, 39, 281], eccentric exercise [151, 200, 510, 541, 546], glyceryl trinitrate patches [295, 489, 490], sclerosing injections [262], aprotinin injections [86, 101, 102], ultrasound [118], and shock wave treatment [11, 121, 135, 222, 332, 513, 545, 567, 571, 604, 605, 610]. What may appear clinically as an acute tendinopathy is actually a well-advanced failure of a chronic healing response in which there is neither histological nor biochemical evidence of inflammation [37]. The available literature

suggests that, in the absence of an overt inflammatory process, there is no rational basis for the use of non-steroidal anti-inflammatory drugs in chronic tendinopathy [407].

In this current concepts review, we report the best available evidence for the management of tendinopathy and provide a comprehensive and up-to-date review of the development of future modalities for treatment.

## I. Non-operative management options:

### *A. Eccentric exercises*

Eccentric exercises have been proposed to promote collagen fibre cross-link formation within the tendon, thereby facilitating tendon remodelling (Table 1). Evidence of histological changes following a program of eccentric exercise are lacking, and the mechanisms by which eccentric exercises may help to resolve the pain of tendinopathy remain unclear.

Eccentric exercises have been proposed to counteract the failed healing response which underlies tendinopathy by promoting collagen fibre cross-linkage within the tendon, thereby facilitating tendon remodelling [545]. The concept of eccentric exercises is based on the structural adaptation of the musculotendinous units to protect them from increased stresses and thus prevent re-injury.

The basic principles in an eccentric loading regime is unknown although it has been speculated that forces generated during eccentric loading are of a greater magnitude than in concentric exercises [528]. It is possible that eccentric exercises do not just exert a beneficial mechanical effect, but also act on pain mediators, decreasing their presence in tendinopathic tendons. Unfortunately, we can only speculate on this effect. Even though microdialysis has shown intratendinous glutamate levels [15], and substance P and neurokinin-1 receptor [25] to be significantly higher in Achilles tendons with painful tendinopathy than in normal pain-free tendons, and treatment with eccentric training has shown good clinical results with diminished tendon pain during activity, *in vivo* results showed that successful treatment with eccentric training was not associated with lowered intratendinous glutamate levels [15]. Also, as the exercise regimen is supposed to produce pain, and, if the patient does not experience pain, load is added to produce pain during the exercise, it is possible that progressive habituation to painful stimuli occurs. Color Doppler sonography demonstrated decreased neovascularisation following eccentric training intervention [473].

Excellent clinical results have been reported both in athletic and sedentary patients [406, 546], although these results were not reproduced by other study groups. In general, the overall trend suggests a positive effect of eccentric exercises, with no reported adverse effects. In one study, combining eccentric training and shock wave therapy produced higher success rates compared to eccentric loading alone or shock wave therapy alone [541].

**Table 1:** Studies on eccentric exercises and tendinopathy. VAS: visual analogue scale; VISA-A: Victorian Institute of Sport Assessment- Achilles; VISA-P: Victorian Institute of Sport Assessment – Patellar; AOFAS: American Orthopaedic Foot and Ankle Society; FFI: Foot Functional Index

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Mafi et al, 2001** [406] | prospective multicenter study (Level I) | 44 patients (22 of 44 patients randomized to eccentric exercises) | Achilles tendon | 12 weeks | Reduction of pain during activity (jogging/walking) (VAS from 69 to 12) | - |
| **Purdam et al, 2004** [518] | non-randomised pilot study (Level II) | 17 patients (22 tendons) | patellar tendon | 15 months | Reduction of pain during activity (VAS score from 74.2 to 28.5); return to previous activity level | - |
| **Roos et al, 2004** [546] | prospective randomized clinical trial (Level I) | 44 patients | Achilles tendon | 52 weeks | pain reduction; improvement of symptoms, function and foot and ankle-related quality of life (Foot and Ankle Outcome Score from 62 to 87) | muscle soreness |
| **Jonsson and Alfredson, 2005** [288] | prospective study (Level I) | 25 patients (19 patellar tendons) | patellar tendon | mean 32.6 months | Reduction of pain (VAS from 73 to 23); improvement of function (VISA-P score from 41 to 83) | - |
| **Young et al,. 2005** [688] | prospective randomised controlled trial (Level I) | 17 patients | patellar tendon | 12 months | Improvement of knee function (VISA-P score from 63 to 87); reduction of tendon pain with activity (VAS from 52 to 30) | - |
| **Bahr et al, 2006** [47] | prospective study (Level I) | 35 patients (40 knees) | patellar tendons | 12 months | Improvement of knee function (VISA –P score from 30 to 70); reduction of pain in standing jump (from 3.9 to 1.7), in counter-movement jump (from 3.9 to 1.8) and in leg press (from 4.0 to 1.3) | - |
| **Jonsson et al, 2006** [290] | Prospective study (Level III) | 9 patients | supraspinatus tendon | 52 weeks | Reduction of pain (VAS from 71 to 18); Constant score from 51 to 80) | - |
| **Sayana and Maffulli, 2007** [562] | Prospective study (Level VI) | 34 patients | Achilles tendon | 12 weeks | Improvement of function VISA-A score from 39 to 50 points) | - |

**Table 1** (*continued*)

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Croisier et al** [140] | Prospective study (Level III) | 92 patients | lateral epicondylar tendons | 9 weeks | Reduction of pain (VAS from 6.9 to 1.2); increase of muscle strength; reduction of disability (Disability questionnaire from 8.5 to 14.4) | - |
| **Frohm et al, 2007** [200] | Prospective, randomised clinical trial (Level I) | 20 patients | patellar tendon | 12 weeks | Improvement in symptoms and function (VISA-P score from 36 to 75 points); reduction of pain (VAS from 5 to 1) | No complication |
| **Nørregaard et al, 2007** [465] | Prospective (Level I) | 45 patients | Achilles tendon | 12 months | Reduction of pain and symptoms; global improvement | - |
| **Petersen et al, 2007** [510] | Randomized controlled clinical trial (Level I) | 100 patients (139 Achilles tendons) (46 tendons randomized to eccentric exercises) | Achilles tendon | 54 weeks | Reduction of pain at rest, during gait, and during sports activities; improvement of function of the hindfoot region (AOFAS hindfoot Scale from 77 to 85); improvement of quality of life | - |
| **Jonsson et al 2008** [289] | Short-term prospective pilot study (Level IV) | 27 patients (34 Achilles tendons) | Achilles tendon | mean 4 months | Reduction fo pain (VAS from 69.9 to 21) | - |
| **Maffulli et al 2008** [403] | Prospective study (Level IV) | 45 patients | Achilles tendon | 12 weeks | Improvement of function (VISA-A) score from 36 to 52 points) | - |
| **de Jonge et al, 2008** [151] | Randomised controlled single blinded clinical trial (Level I) | 58 patients (70 tendons) (34 tendons randomized to eccentric exercises) | Achilles tendon | 12 months | Improvement of function (VISA-A) score from 50 to 76 points); global improvement; | - |
| **Rompe et al 2009** [541] | Randomized controlled trial (Level I) | 68 patients (34 of 68 randomized to eccentric exercises) | Achilles tendon | 4 months | Improvement of function (VISA-A) score from 50 to 73 points); reduction of load-induced pain (Pain rating from 7 to 4 points); global improvement (Likert scale of 1 or 2 points) | ache in the calf after eccentric loading |
| **Kulig et al 2009** [326] | Case Series (Level IV) | 10 patients | tibialis posterior tendons | 6 months | Improvement in symptoms and function (FFI from 31.1 to 10.9); reduction of pain after 5-min walk test (VAS from 21.6 to 7.4) | - |

*B. Extracorporeal shock-wave therapy*

Extracorporeal shock wave therapy (ESWT) to address the failed healing response of a tendon is becoming more widely used among the medical community (Table 2) [545]. Typical characteristics are high peak-pressure amplitudes (500 bar) with rise times of < 10 ns, a short lifecycle (10 ms) and a frequency spectrum (16 Hz–20 MHz) ranging from the audible to the far ultrasonic level [542]. This rapid rise is followed by periods of pressure dissipation and negative pressure before gradually returning to the ambient pressure. The shock wave entering the tissue may be reflected or dissipated, depending on the properties of the tissue. The energy of the shock wave may act through mechanical forces generated directly or indirectly via cavitation [544]. The rationale for the clinical use of ESWT is stimulation of soft tissue healing and inhibition of pain receptors.

There is no consensus on the use of repetitive low-energy ESWT, which does not require local anaesthesia, and on the use of high-energy ESWT, which requires local or regional anaesthesia [544]. In several well conducted randomized controlled trials, low energy ESWT was been administered once a week for three or four consecutive weeks, with final assessment undertaken 12 weeks after the last ESWT session [541, 545]. At 4-month follow-up, eccentric loading and low-energy SWT showed comparable results [542], while eccentric loading alone was less effective when compared with a combination of eccentric loading and repetitive low-energy shock-wave treatment [541].

High energy ESWT is instead administered only once.

Low-energy shock wave therapy in tendinopathy has been proposed to stimulate soft tissue healing and inhibits pain receptors [540, 541, 543, 544]. Low energy shock wave therapy or eccentric training for the management of Achilles tendinopathy produced comparable results in a randomized controlled trial [545], and both management modalities showed outcomes superior to no intervention [545]. However, results of low energy shock wave therapy were also disappointing in another study [137].

*C. Use of injectable substances*

A wide variety of substances have been injected and are routinely injected in and around tendons.

1. High volume injections – normosaline solution, corticosteroids and anaesthetics

Neo-vascularisation is a characteristic feature of Achilles and patellar tendinopathy, generally accompanied by nerve in-growth, and generally it is not present in patients without tendon pathology [18, 325]. The ingrowth of new blood vessels and associated nerves from the ventral side of the tendon may be a source of pain [474]. Histopathological studies showed immunoreactions for neurokinin-1 receptor and alpha1-adrenoreceptor in biopsies performed in the ventral area of the tendinopathic Achilles [24], and patellar tendon [148] as well as elevated levels of the neurotransmitter glutamate and the presence of its receptor, N-methyl-d-aspartate receptor type 1 [147, 148, 564].

**Table 2:** Studies on extracorporeal shock-wave therapy and tendinopathy- VAS: visual analogue scale; SPADI: Shoulder Pain and Disability Index; CMS: Constant and Murley Scale; EQ-5D: EuroQol-5D; DASH: Disabilities of the Arm, Shoulder and Hand

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Schmitt J et al 2001** [567] | Prospective, controlled, randomised study (Level I) | 40 patients | tendinopathy of the supraspinatus | 12 weeks | Increase in function (Constant score from 40.70 to 66.50); reduction of pain during rest (VAS from 5.35 to 2.30) and pain during activity (VAS from 7.75 to 4.85) | No side-effects |
| **Speed et al 2002** [604] | Double blind placebo randomised controlled trial (Level I) | 75 patients | lateral epicondylitis | 3 months | Reduction of pain (VAS from 73.4 to 47.9) and night pain (VAS from 40.4 to 33.5); improvement of function | withdrawn after 2 treatments due to worsening of symptoms |
| **Speed et al 2002** [605] | Double-blind, randomised, controlled trial (Level I) | 74 patients | chronic tendinopathy of the rotator cuff | 6 months | Reduction of pain and improvement in shoulder function SPADI) from 53.6 to 24.1); reduction of night pain (VAS from 60.9 to 27.3) | No adverse effects |
| **Jakobeit et al. 2002** [280] | Prospective study (Level IV) | 80 patients | chronic calcareous tendinopathy of the shoulder rotator cuff | 4 weeks | Reduction or absence of pain at rest, pain during night sleep, pressure pain, pain in movement and pain during shoulder stress; reduction of restriction of shoulder movement (active abduction from 80% to 10%; active antiversion from 59% to 2%; clasping of hands to the nape of the neck from 65% to 11%; hands clasped in the small of the back from 46% to 9%); Reduction or complete resorbement of calcifications | - |
| **Cosentino et al 2003** [135] | Single blind randomised study (Level I) | 70 patients | calcifying tendinopathy of the rotator cuff | 6 months | Decrease of pain and increase in shoulder function (Constant score from 45 to 76) | - |
| **Gerdesmeyer et al 2003** [222] | Double-blind, randomized, placebo-controlled trial (Level I) | 144 patients | calcifying tendinopathy of the rotator cuff | 12 months | Improvement of shoulder function (CMS) from 60 to 91); reduction of pain (VAS from 6.5 to 0.9) | Petechiae, bleeding, hematoma and erythema after treatment; no clinically adverse effects (including neurologic disorders, tendon rupture, infection, bone edema, aseptic necrosis, or muscle hematoma) |

**Table 2** (*continued*)

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Chung and Wiley 2004** [121] | Double-blind randomized controlled trial (Level I) | 60 patients | lateral epicondylitis | 8 weeks | Reduction of overall pain (VAS from 3.9 to 2.0), resting pain (VAS 1.2 from to 1.0), night pain (VAS from 1.3 to 0.4), activity pain (VAS from 5.2 to 2.4); improvement of quality life (EQ5D thermometer from 81 to 84); increase of maximum pain-free grip strength (from 24.7 to 30.0 kg) | Nausea during therapy; achiness after therapy; soreness after therapy; increased pain symptoms after therapy |
| **Peters et al 2004** [508] | Prospective study (Level 1) | 90 patients | calcific tendinopathy of the shoulder | 6 months | Reduction of pain; decrease of calcifications | Transitory reddening of the skin; pain; small haematomas |
| **Chung et al. 2005** [122] | Prospective cohort (Level II) | 60 patients | lateral epicondylitis | 12 months | Reduction of overall pain (VAS from 3.9 to 0.3), resting pain (VAS from 1.2 to 0.05), night pain (VAS from 1.3 to 0.3), activity pain (VAS from 5.2 to 0.2); improvement of quality of life; increase of maximum pain-free grip strength | - |
| **Pettrone and McCall 2005** [513] | Randomized double-blind controlled trial (Level I) | 108 patients | lateral epicondylitis | 12 weeks | Reduction of pain (VAS from 74 to 37.6); improvement of function (Activity score from 7.7 to 3.5); improvement of grip strength (from 71 to 87.1 lb) | Pain; nausea; local reaction; sweating; dizziness; hypertonia; hypesthesia; paresthesia; joint stiffness; myalgia; tremor; vasodilation; pallor |
| **Lebrun 2005** [332] | Randomized double-blind controlled trial (Level I) | 60 patients | lateral epicondylitis | 8 weeks | Reduction of overall pain; improvement of quality of life; increase of pain-free grip strength | - |
| **Moretti et al 2005** [449] | Prospective study (Level IV) | 54 patients | rotator cuff calcifying tendinopathy | 6 months | Improvement of shoulder function (Constant score from 24.5 to 68.2); reduction of pain (VAS from 4.5 to 1.92) | No systemic or local complications |
| **Furia 2005** [207] | Prospective study (Level III) | 36 patients | chronic lateral epicondylitis | 12 weeks | Reduction of pain (VAS from 8.0 to 2.5); improvement of function (RAND 36-Item Health Survey (Physical Functioning) score from 65.6 to 88.0) | no complications |

**Table 2** (*continued*)

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Furia 2006** [208] | Case control study (Level III) | 68 patients | Achilles tendon | 12 months | Reduction of pain (VAS from 7.9 to 2.8); reduction or absence of symptoms (Roles and Maudsley scale) | pain during the treatment; transitory reddening of the skin; transitory numbness on the plantar aspect of the heel |
| **Albert et al 2007** [11] | prospective randomised trial (Level I) | 80 patients | rotator cuff calcifying tendinopathy | 3 months | Improvement of function (Constant and Murley score from 50.7 to 63.2); reduction of pain | No serious adverse events |
| **Vulpiani et al 2007** [658] | prospective study (Level IV) | 73 patients (83 knees) | Patellar tendinopathy | 24 months | Reduction of pain (VAS from 7.1 to 1.35); global improvement (subjective clinical evaluation from 1.21 to 0.31) | - |
| **Hsu et al, 2008** [267] | Prospective study (Level I) | 33 patients | calcific tendinopathy of the shoulder | 12 months | Reduction of pain (VAS from 7.2 to 1.3); improvement of function (Constant score from 57.3 to 88); absence or decrease of calcium deposits | Local erythematous changes; local discomfort |
| **Staples et al. 2008** [610] | Double-blind, randomized, placebo-controlled trial (Level I) | 68 patients | lateral epicondylitis (tennis elbow) | 6 months | Reduction of pain (Pain Index mean change 31.7); improvement of function (Function Index mean change 9.2, Dash Function mean change 21.0, Dash Sport mean change 34.9, Dash Work mean change 27.9); increase of pain-free grip (mean change 0.43) and maximum grip strength (mean change 0.23) | Pain or tenderness in the arm; burning sensation |
| **Vulpiani et al, 2009** [657] | observational study (Level IV) | 105 patients (127 tendons) | Achilles tendon | 24 months | Reduction of pain (VAS from 7.49 to 2.6); improvement of function | - |
| **Schofer et al. 2009** [571] | prospective, randomised, controlled study (Level I) | 40 patients | rotator cuff | 12 months | Increase in function (Constant score); subjective improvement; reduction of pain | - |

The hypothesised rationale behind the management modality was that the high volume injections of normosaline solution, corticosteroids and anaesthetics would produce local mechanical effects causing new blood vessels to stretch, break or occlude. By occluding and possibly breaking these vessels, the accompanying nerve supply would also be damaged either by trauma or ischemia, therefore decreasing the pain in patients with resistant Achilles tendinopathy.

Preliminary studies showed that high volume injection of normosaline solution, corticosteroids and anaesthetics reduces pain and improves short and long-term function in patients with Achilles [108] and patellar tendinopathy [139], regardless of their symptoms (Table 3). High volume injection is safe and relatively inexpensive, with the potential to offer an alternative management option to operative treatment resulting in a quicker return to sport [271]

Hydrocortisone acetate is used in the high volume injections, primarily to prevent an acute mechanical inflammatory reaction produced by the large amount of fluid injected in the proximity of the tendon. The injection is performed under ultrasound guidance, so that corticosteroid has no direct action on the tendon itself. The role of corticosteroid in the management of tendinopathy is still debated. Meta-analysis of the effects of corticosteroids has shown that published data are insufficient to determine the risk of rupture following corticosteroid injections [590], and we do not advocate their intra-tendinous injection [271]

**Table 3:** Studies on high volume injections and tendinopathy

| Study (references) | Level of evidence | N° patients | Tendon/ injected substance | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Crisp et al 2008** [139] | Retrospective (Level IV) | 9 patients | patellar tendon. The injection contained 10 ml 0.5% Bupivacaine, 25 mg Hydrocortisone, and between 12 and 40 ml normosaline | 9 months | Reduction of pain; improvement in function | 1 patient failed to respond to the therapy; 3 patients experienced partial recurrence of patellar tendinopathy |
| **Chan et al 2008** [108] | Retrospective (Level IV) | 30 patients | Achilles tendon. The injection contained 10 ml of 0.5% Bupivacaine Hydrochloride, 25 mg Hydrocortisone acetate, and up to 40 ml of injectable normal saline | Mean 8 months | Pain and functional improvement | - |

2. Platelet-rich plasma

Platelet-rich plasma (PRP) is a bioactive component of whole blood, which is now being widely tested in different fields of medicine for its possibilities in aiding the regeneration of tissue with poor healing potential [193, 244, 322, 552, 554].

The use of PRP to help wound healing has been proposed since the early 1980s [311]. Its use in orthopaedic surgery began during this decade especially for augmentation of bone grafting, even though to date no definitive evidence is available for its use to improve bone healing. The use of PRP to favour tendon healing has been advocated only recently [442, 443, 553]. PRP, in general, has a higher concentration of platelets compared with blood.

Dense granules may play a role in tissue modulation and regeneration by releasing their content of adenosine, serotonin, histamine, and calcium. The alpha granules release the transforming growth factor-b, platelet-derived growth factor, and vascular endothelial growth factor, with concentrations increasing linearly with increasing of platelet concentration. The released cytokines bind to transmembrane receptors on the surface of local or circulating cells and induce intracellular signalling. This may result in the production of proteins responsible for cellular chemotaxis, matrix synthesis, and proliferation [443].

Tendon healing occurs through 3 overlapping phases (inflammation, proliferation, and remodelling), which are controlled by a variety of growth factors [443, 581, 584, 585]. The rationale for the use of PRP to promote tendon healing is the high content of these cytokines and

**Table 4:** Studies on platelet-rich plasma and tendinopathy. VAS: visual analogue scale; SF-36:Short Form (36) Health Survey; EQ-5D: EuroQol-5D; VISA-A: Victorian Institute of Sport Assessment- Achilles

| Study (references) | Level of evidence | Tendon | N° patients | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Mishra and Pavelko 2006** [442] | Prospective cohort study (Level II) | Common extensor or flexor tendon | 20 patients | mean 25.6 months (range 12-38 months) | reduction of visual analog pain score (93% of treated patients) | No complications |
| **Filardo et al 2009** [186] | Prospective (Level IV) | Patellar tendon | 15 patients | 6 months | statistically significant improvement in Tegner score, EQ VAS score and pain level | No complications |
| **Kon et al 2009** [322] | Prospective (Level IV) | Patellar tendon | 20 male athletes | 6 months | Improvement in Tegner, EQ VAS and SF 36 scores | No complications related to the injections or any severe adverse events |
| **De vos et al 2010** [154] | Prospective randomized study (Level 1) | Achilles tendon | 54 randomized patients | 24 weeks | The mean VISA-A score improved in both groups. The increase was not different between both groups | No complications |

cells in hyperphysiologic doses of PRP. Several studies are ongoing worldwide on the application in PRP for tendon healing, even though the exact mechanisms by which PRP promote tendon healing is not still clear (Table 4). One of the main advantages is that PRP is autologous and is prepared at the time of treatment (point of care), and therefore it has an excellent safety profile. De vos et al performed a stratified, block-randomized, double-blind, placebo-controlled trial at a single center on 54 patients with Achilles tendinopathy undergoing exercises (usual care) with either a PRP injection (PRP group) or saline injection (placebo group). The authors concluded that among patients with chronic Achilles tendinopathy who were treated with eccentric exercises, a PRP injection compared with a saline injection did not result in greater improvement in pain and activity.

3. Autologous blood injection

An injection of autologous blood has been reported for the management of tendinopathy [170] with the aim of providing cellular and humoral mediators to induce healing in areas where the healing response has failed (Table 5). The use of autologous blood injection is thought to lead to tendon healing through collagen regeneration and the stimulation of a well-ordered angiogenesic response. It is hypothesized that transforming growth factor-β and basic fibroblast growth factor carried in the blood will act as humoral mediators to induce the healing cascade [279, 519]. Although the results of laboratory studies are encouraging, they have always used healthy tendons or surgically induced lesions, given the lack of a good experimental model for tendinopathy. At present, it is unclear whether these results can be extrapolated to tendinopathic tendons. So called needling of the tendon has been described in conjunction with the use of autologous blood. In this respect, however, it could be difficult to distinguish between the effects of needling and the effect of autologous blood injection [519].

4. Polidocanol

In patients with chronic painful Achilles tendinopathy there is neovascularisation outside and inside the ventral part of the tendinopathic area [312, 313]. Local anaesthetic injected in the area of neovascularisation outside the tendon may result in a pain-free tendon, indicating that this area is involved in pain generation. These are the bases for the injection of the sclerosing substance Polidocanol (Aetoxisclerol®, Kreussler, Germany) under ultrasonography and colour Doppler-guidance in the area with neovessels outside the tendon [14, 16, 17, 19, 288, 471].

Injections with Polidocanol showed the potential to reduce tendon pain during activity in patients with chronic painful mid-portion Achilles tendinopathy in a randomized controlled trial (Table 6) [17].

**Table 5:** Studies on injection of autologous blood and tendinopathy. VAS: visual analogue scale; SF-36:Short Form (36) Health Survey; EQ-5D: EuroQol-5D; VISA-P: Victorian Institute of Sport Assessment – Patellar

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Edwards and Calandruccio 2003** [170] | Prospective (Level IV) | 28 patients | extensor carpi radialis brevis (ECRB) | 9.5 months (range, 6-24 months) | decrease in pain score (from 7.8 to 2.3) and Nirschl score (from 6.5 to 2.0) | - |
| **Suresh SP et al 2006** [618] | Prospective (Level IV) | 20 patients | common flexor origin of elbow | 10 months | decrease in VAS pain and in the modified Nirschl scores (from 6 to 1); reduction of hypo-echoic changes in the flexor tendon; reduction of neovascularity | no infection, neurovascular damage or rupture of the tendon |
| **Connell et al 2006** [129] | Retrospective(Level IV) | 35 patients | extensor tendon origin of elbow | 6 months | reduction in VAS pain (from 9 to 0) and in Nirschl scores (from 6 to 0); reduction in the total number of interstitial cleft formations and anechoic foci; reduction in tendon thickness; reduction of hypoechoic changes and neovascularity | no infection, neurovascular damage or rupture of the tendon |
| **James et al 2007** [282] | Prospective cohort study (Level IV) | 44 patients (47 knees) | Patellar tendon | 14.8 months (range 6 to 22 months); 21 patients (22 knees) | reduction in overall tendon thickness and in the size of the area of tendinopathy; improvement in VISA-P score (from 39.8 to 74.3) | No complication |
| **Moon et al 2008** [448] | Prospective (Level IV) | 24 patients (26 elbow) | insertion area of the extensor carpi radialis brevis (ECRB) and flexor origin tendon | 6 months | improvement in VAS and Mayo elbow performance scores | No complication |

**Table 6:** Studies on polidocanol injections and tendinopathy. VAS: visual analogue scale; VISA-P: Victorian Institute of Sport Assessment – Patellar

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Ohberg and Alfredson 2002** [471] | Prospective (Level IV) | 10 patients | Achilles tendon | 6 months | reduction of pain during activity (VAS-scale decreased from 74 to 8) | no side effects of the treatment |
| **Ohberg and Alfredson 2003** [472] | Prospective (Level IV) | 11 patients | Achilles tendon | mean 8 months | Reduction of pain during tendon-loading activity (VAS-scale decreased from 82 mm to 14 mm) and reduction of neovascularisation | no side effects |
| **Alfredson and Ohberg 2005** [16] | Prospective(Level IV) | 15 patients (15 patellar tendons) | patellar tendons | Mean 6 months | reduction of pain (VAS from 81 to 10) | 3 tendons presented remaining neovascularisation after treatment |
| **Alfredson and Ohberg 2005** [17] | Prospective (Level IV) | 20 patients | Achilles tendon | mean 3 months | reduced level of tendon pain, neo-vascularisation absent after treatment | no adverse events or side effects |
| **Zeisig et al 2006** [693] | Prospective (Level IV) | 11 patients (13 elbows) | Extensor tendon origin of elbow | 8 months | reduction of pain (VAS from 75 to 34); increase of maximal grip strength (from 29 to 40 kg) | no complications related to the treatment |
| **Alfredson et al 2006** [14] | Prospective (Level IV) | 14 patients (14 shoulders) | supraspinatus tendon | Mean 8 months | reduction of pain (VAS-scale decreased from 79 to 21) | 5 patients with a poor result of the polidocanol injections |
| **Hoksrud et al 2006** [262] | Randomized controlled trial/cross-over study (Level I) | 33 patients (42 tendons) | Patellar tendon | 12-months | improvement of pain level and function (VISA-P score from 54 to 75) | No adverse events or side effects |
| **Lind et al 2006** [347] | Prospective (Level IV) | 42 patients | Achilles tendon | 24 months | reduction in VAS (from 75 to 7) and in the mean mid-portion tendon thickness (from 10 to 8 mm) | no adverse events or side effects |
| **Alfredson et al 2007** [19] | Prospective (Level IV) | 20 patients | Achilles tendon | 6 months | reduction of tendon pain level (VAS from 76 to 24) | – |

**Table 6** (*continued*)

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Willberg et al 2008**[675] | Prospective (Level IV) | 48 patients (52 Achilles tendons) | Achilles tendon | mean 14 months | reduction of VAS scale (from 66 to 24) | no adverse events or side effects |
| **Hoksrud et al 2008**[263] | Cohort study (Level III) | 63 patients (79 Achilles tendons) | Achilles tendon | 15 months | improvement of function | - |
| **Zeisig et al 2008**[694] | Follow-up study (Level IV) | 25 patients (28 elbow tendons) | Extensor tendon origin of elbow | 24 months | structural tendon changes and high blood flow at inclusion | - |
| **Clementson et al 2008**[123] | Retrospective study (Level IV) | 28 patients (29 Achilles tendons) | Achilles tendon | between 6–12 months | good or excellent in self-assessment questionnaire or phone interview | 8 patients experienced discomfort during the treatment; 1 patient stopped treatment because of affection of the sural nerve with parestesias and numbness |

In Achilles and patellar tendinopathy, there is evidence of neural in-growth in conjunction with neo-vascularisation. Injections of Polidocanol close to the tendon seem to be remarkably safe.

In 150 patients managed with Polidocanol for Achilles tendinopathy, two complications were experienced. One patient who had insertional Achilles tendinopathy sustained a total rupture in the proximal part of the tendon at the end of an 800-m running race, and one patient managed with Polidocanol sustained a partial rupture in the midportion of the tendon where he previously had received four intratendinous corticosteroid injections [13].

5. Intratendinous injections of corticosteroids

The use of corticosteroid injections is highly controversial [117, 246, 254, 433, 488, 529]. There is a lack of good quality research data to support the widespread use of these drugs. In patients, there are numerous case reports of tendon rupture after corticosteroid injections [187, 310]. Animal studies have suggested that local corticosteroid injections may lead to a reduction in tendon strength [298], but this finding is not universal [422].

At present, there is insufficient evidence from which to draw firm conclusions on the utility of local corticosteroid treatments for Achilles tendinopathy (Table 7). Three randomized controlled trials [144, 199, 460] showed different results with the use of local corticosteroids on healing, with two studies reporting some benefit [199, 460] and the other showing none [144]. A meta-analysis of the effects of corticosteroid injections has shown little benefit [590]. The safety of using corticosteroid injections can be enhanced with the use of ultrasound imaging. In the high volume injection technique, the needle is kept extra-tendinous and outside the peritendinous space [227], so that the fluid is injected only in Kager's triangle (for the Achilles tendon) or in Hoffa's body (for the patellar tendon)

6. Aprotinin

Aprotinin is an 85 amino-acid, 65 kD basic polypeptide extracted from bovine lungs. It is a broad spectrum serine protease inhibitor, with particular inhibition of plasmin (along with trypsin and kallikrein) [150, 171, 477]. It forms reversible competitive bonds with certain enzymes, inhibiting their proteolytic action and vasoactive effects in the early stages of inflammation [150, 171]. It may block matrix metalloproteinases (MMPs), including MMP-1, MMP-8 and MMP- 13 (collagenases) and MMP-2 and MMP-9 (gelatinases), either directly or via inhibition of plasminogen and plasmin [150, 171].

Aprotinin has primarily been used in medicine to promote soft tissue healing after an operation (as a component of ''fibrin glue'') [321, 477], and to prevent blood loss during major operative procedure [127]. The major side effect is anaphylaxis, which is particularly seen after repeated use of the drug [164, 478]. Aprotinin has been used for over 35 years as an offlabel injection for management of tendinopathy [38, 101, 102, 218, 402, 536, 548]. The rate of allergic reaction when using repeat injections of aprotinin (bovine-derived) is higher than

**Table 7:** Studies on intratendinous injections of corticosteroids and tendinopathy. VAS: visual analogue scale; PRFEQ: Patient Related Forearm Evaluation Questionnaire; DASH: Disabilities of the Arm, Shoulder and Hand; SPDI: Shoulder pain and disability index

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Saartok and Eriksson 1986** [550] | Randomized single-blind pilot study (Level I) | 21 patients (11 of 21 randomised to corticosteroids injections) | Tennis elbow (lateral epicondylitis) | 2 weeks | Reduction of pain at rest and during daily activity; reduction of limitation of extension; improvement of grip strength | No side effects |
| **Anderson et al 1991** [23] | Prospective study (Level IV) | 55 patients | Extensor pollicis brevis tendon | 4 year | Reduction of pain; Finkelstein test negative | Pain at the injection site (18 of 55 patients); inflammatory flare reaction (pain, swelling, heat) (5 of 55 patients); ecchymosis at the injection site (9 of 55 patients); radial nerve paresthesia, temporary (2 of 55 patients); vasovagal reaction (2 of 55 patients); subcutaneous fat atrophy (16 of 55 patients) |
| **Price et al 1991** [516] | Prospective, randomized double-blind study (Level I) | 88 patients (59 of 88 randomised to corticosteroids injections) | Tennis elbow | 24 weeks | reduction of pain (VAS from 47 to 18) and tenderness (Tenderness score from 2.1 to 0.6) | skin atrophy |
| **Vecchio et al 1993** [653] | Prospective double-blind trial (Level I) | 28 patients (28 of 55 randomised to corticosteroids injections) | Rotator cuff | 12 weeks | Reduction of pain; increase of active abduction and active external rotation; improvement of total resisted movement score | mild transient post-injection ache |
| **Sölveborn et al 1995** [596] | Prospective, randomized, double-blind study (Level 1) | 109 patients | Extensor carpi radialis brevis tendon | 1 year | reduction of pain in long-term period (VAS from 44 to 18) | No side effects |
| **Verhaar et al 1996** [656] | Prospective randomized trial (Level 1) | 106 patients (42 of 106 randomised to corticosteroids injections) | Extensor digi-torum tendon and extensor carpi radialis brevis tendon | 52 weeks | increase in grip strength; reduction of pain in short-term period | No side effects (infections, skin hypopigmentation) |

**Table 7** (*continued*)

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Stahl and Kaufman 1997** [609] | Prospective, randomized double-blind study (Level 1) | 58 patients (30 of 60 elbows randomised to corticosteroids injections) | flexor-pronator tendon origin of elbow | 12 months | reduction of pain in short-term period | No local complications |
| **Hay et al 1999** [253] | Multicentre randomised controlled trial (Level 1) | 164 patients (53 of 164 elbows randomised to corticosteroids injections) | Tennis elbow (lateral epicondylitis) | 12 months | Reduction of pain severity and disability; improvement of function; increase of pain free grip strength in affected arm | Local skin atrophy (1 of 53 patients) |
| **Smidt.et al 2002** [594] | Prospective, randomized controlled study (Level 1) | 185 patients (62 of 185 randomized to corticosteroids injections) | Tennis elbow (lateral epicondylitis) | 52 weeks | General improvement; reduction of pain and functional disability; increase of pain-free grip strength | Increased pain <1 day (6 of 62 patients); increased pain >1 day (10 of 62 patients); radiating pain to forearm or upper arm (17 of 62 patients); facial flush (2 of 62 patients); skin irritation (3 of 62 patients); red swollen elbow (2 of 62 patients); change of skin colour (7 of 62 patients) |
| **Crowther et al 2002** [141] | Prospective, randomised study (Level 1) | 93 patients | Extensor tendon origin of elbow | 3 months | reduction of pain (VAS from 67 to 12) | No side effects |
| **Koenig et al. 2004** [315] | Uncontrolled, prospective study (Level IV) | 5 patients (6 tendons) | Achilles tendon | 3 months | reduction of pain at rest and pain at activity; reduction of intratendinous hyperaemia | - |
| **Gill et al 2004** [227] | Retrospective cohort study (level of evidence IV) | 43 patients | Achilles tendon | Mean 38 months | Clinical condition improved in 40% of patients, unchanged in 53% of patients and in 7% of patients worsen | No major complications (tendon rupture) and one minor complication (purple skin discoloration) |

**Table 7** (*continued*)

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Lewis et al 2005** [343] | Randomized controlled trial (Level 1) | 164 patients (53 of 164 randomized to corticosteroids injections) | Tennis elbow (lateral epicondylitis | 5 days | reduction of pain after 24 hours of treatment | - |
| **Bisset et al 2006** [70] | Single blind randomized controlled trial (Level 1) | 198 patients (65 of 198 randomised to corticosteroids injections) | Tennis elbow | 52 weeks | global improvement, grip force augmentation and pain reduction | Pain after treatment (12 of 65 patients); hypopigmentation (2 of 65 patients); atrophy of subcutaneous tissue (1 of 65 patients) |
| **Tonks et al 2007** [639] | Prospective randomised controlled trial (Level 1) | 48 patients (12 of 48 randomised to corticosteroids injections) | common extensor origin | 7 weeks | Increase of pain free grip strength and extensor weight strength; improvement of score of the PRFEQ | skin depigmentation and atrophy |
| **Peters-Veluthamaningal et al 2008** [509] | Randomised placebo controlled double-blinded trial (Level 1) | 50 patients (25 of 50 randomised to corticosteroids injections) | flexor tendon | 12 months | Reduction of frequency of triggering; reduction of pain | hot flushes (9 patients); steroid-flare (6 patients) |
| **Lindenhovius et al 2008** [349] | Prospective, Double-Blind, Randomized Clinical Trial (Level 1) | 64 patients (31 of 33 randomised to corticosteroids injections) | Lateral elbow | 6 months | reduction of pain (VAS score from 5.8 cm to 2.4 cm; DASH score from 31 points to 18; increase of grip strength (percentage of grip strength (involved/noninvolved) from 83% to 98%) | Slight discoloration of skin around the injection site (1 patient) |
| **Ekeberg et al. 2009** [174] | Double blind randomised clinical trial (Level 1) | 106 patients (53 of 106 randomised to corticosteroids injections) | Rotator cuff | 6 weeks | Improvement in shoulder pain (SPDI from 53 to 29; Western Ontario rotator cuff index from 45 to 67; abduction from 131 to 141; flexion from 151 to 156; pain at rest from 6 to 3; pain in activity from 6 to 2 | Post-injection pain in the shoulder |

for most medications and this represents a major factor to consider when choosing this drug [477, 478]. If aprotinin works simply as a form of prolotherapy, it would be a better choice to use dextrose or autologous blood for treatment of tendinopathy. However, if aprotinin works specifically as a collagenase inhibitor, then it may have advantages over more inert substances. This is an important question with mixed results demonstrated to date in the randomised controlled trials (Table 8) [86, 102].

Brown et al [86] conducted a randomised controlled trial to compare aprotinin and exercises with placebo and exercises. They found no statistically significant improvement in the aprotinin group over placebo at any follow up visit for either the primary or secondary outcome measures. However, a beta error was admitted by the authors, as the lack of statistical significance could be due to the small sample size.

## II. Operative treatment

The objectives of operative treatment are to excise fibrotic adhesions, remove or debride areas of failed healing, restore vascularity, and possibly stimulate viable cells to initiate protein synthesis and to promote healing. Recent studies show that multiple longitudinal tenotomies trigger neoangiogenesis in the Achilles tendon, with increased blood flow [389]. This would result in improved nutrition and a more favourable environment for healing.

Multiple percutaneous longitudinal tenotomies can be performed when conservative management has failed in patients who have isolated tendinopathy with no involvement of the paratenon and a well-defined nodular lesion less than 2.5 cm long [400]. This procedure may be ultrasound guided to confirm the precise location of the area of tendinopathy [400, 629, 630]. It is a simple procedure and can be performed in an ambulatory setting under local anaesthesia without a tourniquet.

Percutaneous longitudinal ultrasound-guided internal tenotomy of the Achilles tendon can be also performed on an outpatient basis. It, however, requires the use of high-resolution ultrasound to properly locate the tendinopathic area and to place the initial stab incision [400, 629, 630]. Complications (wound healing) are minimal and led to no long-term morbidity. The technique is not as effective in patients with pantendinopathy.

### *A. Radiofrequency Microtenotomy*

Radiofrequency microtenotomy is a safe and effective procedure to manage patients with chronic tendinopathy (Table 9). It is a technically simple procedure to perform and has been proposed to produce a rapid and uncomplicated recovery [467, 620, 622, 623]. It is hypothesized that the mechanism of action may be to induce acute degeneration and/or ablation of sensory nerve fibers. Early degeneration followed by later regeneration of nerve fibers after bipolar radiofrequency treatment may explain long-term postoperative pain relief [467, 620, 622, 623].

**Table 8:** Aprotinin
VISA-A: Victorian Institute of Sport Assessment – Achilles

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Capasso et al 1993** [101] | Prospective study (Level 1) | 97 patients (77 of 97 patients randomised to aprotinin injections) | Achilles tendinopathy | 1 year | >75% excellent and good results | No allergic reactions |
| **Capasso et al 1997** [102] | Prospective, randomised, double blind trial (Level 1) | 116 patients (40 of 116 patients randomised to aprotinin injections) | patellar tendinopathy | 12 months | 72% excellent and good results | Burning sensation; itching |
| **Orchard et al 2005** [476] | Retrospective study (Level IV) | 121 patients | Achilles mid-substance Tendinopathy, Achilles insertional tendinopathy, patellar tendinopathy, medial hamstring insertional tendinopathy, proximal hamstring origin tendinopathy and lateral epicondylitis | Mean 9.3 Month | Improvement of the patients' conditions (69% of patients improved) | Itch; rash; sweating; post-injection pain; nausea/abdominal cramps; systemic allergic reaction; headache; tendon damage; post-injection bleeding |
| **Rochcongar et al 2005** [536] | non-randomized prospective study (Level II) | 164 patients (209 tendinopathy) | Achilles tendinopathy | - | 82.4% excellent and good results | local allergic reactions |
| **Brown et al 2006** [86] | Prospective, randomised, double blind, placebo controlled trial (Level I) | 26 patients (33 affected tendons) | Achilles tendinopathy | 12 months | Improvement of performance (VISA-A from 8.5 to 36.3); return to sports (from 0% to 77%); improvement of function (number of hops to pain from 1.5 to 7.4, number of single leg heel raises to pain from 0.7 to 4.3); reduction of pain (patient Rating from 2.0 to 4.9) | Itch; headache. No allergic reactions, infections, ruptures, or other side effects |

**Table 8** (*continued*)

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Orchard et al 2008** [478] | Retrospective historical cohort study (Level IV) | 307 patients (381 cases of tendinopathy) | | Mean 11 months | Improvement of the patients' conditions (64% - 72%) of patients improved) | Itch; bleeding; rash; systemic allergic reaction; nausea; sweating; post-injection pain; headache; tendon damage |
| **Orchard et al 2008** [477] | Case series (Level IV) | 430 patients | patellar and Achilles tendinopathies | Mean 12.2 months | Improvement of symptoms (67% of patients) | Itch (25%); rash (7%); sweating (4%); nausea (4%); allergic reaction (4%); postinjection pain (4%); headache (3%); tendon damage |

**Table 9:** Studies on radiofrequency-based microtenotomy and tendinopathy. DASH: Disabilities of the Arm, Shoulder and Hand; ASES: American Shoulder and Elbow Surgeons; UCLA: University of California, Los Angeles;VAS: VAS: visual analogue scale

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Tasto et al 2005** [623] | Prospective, nonrandomized consecutive case series (Level IV) | 13 patients | common extensor tendon origins of the elbow | 24 months | Pain reduction; upper limb DASH score and SF-36 questionnaire improvement | No perioperative or postoperative complications or adverse events |
| **Taverna et al 2007** [624] | Randomized controlled study (Level I) | 60 patients | supraspinatus tendon | 12 months | Pain score 1.0; ASES score greater than 90; Constant score greater than 80; UCLA questionnaire greater than 30 | No perioperative or postoperative complications or adverse events |
| **Liu et al 2008** [352] | Prospective (Level IV) | 17 patients | achilles tendon | Not reported | Reduction of VAS score (from 8.7 to 1.6) | No postoperative complications |
| **Meknas et al 2008** [430] | Randomized controlled trial (Level I) | 24 patients | extensor tendon of the elbow | 18 months | Grip strength improvement; functional score increase | No complications or adverse events |

*B. Neovessel destruction*

Pathologic nerve ingrowth accompanies pathological neovascularization in the tendinopathic tendon, and it has been considered as a possible cause of the pain. Some authors have attempted to disrupt the abnormal neoinnervation to interfere with the pain sensation caused by tendinopathy. Endoscopy [631, 649-652, 673], electrocoagulation [76] and minimally invasive stripping have been described to achieve this aim. Endoscopy allows direct visualization of the area of tendinopathy, and allows to use motorized instruments or diathermy to destroy neovessels.

*Endoscopy – assisted treatment*

Tendoscopy may allow endoscopic access to several tendons, including posterior tibial tendon [650], the peroneal tendons [573, 649] and Achilles tendon [572, 611, 631, 652] (Table 10). This operative technique provides access to the posterior aspect of the ankle and subtalar joints. Also extra-articular structures of the hindfoot such as the os trigonum, flexor hallucis longus (FHL) and the deep portion of the deltoid ligament can be accessed [651].

Thermann et al [631] described a different technique of endoscopic debridement of the ventral neovascularized area, the peritenon and the Achilles tendon, with good short term clinical results in 8 patients.

**Table 10:** Studies on endoscopy and tendinopathy. UCLA: University of California, Los Angeles; VAS: VAS: visual analogue scale; ADLs: MCEPI: Mayo Clinic Elbow Performance Index; PRTEE: The Patient-Rated Tennis Elbow Evaluation; ASES: American Shoulder and Elbow Surgeons; JOA: Japanese Orthopedic Association

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **van Dijk et al 1997** [650] | Prospective study (Level IV) | 16 patients | posterior tibial tendon | 12 months | Absence of symptoms; improvement of function | no complications |
| **Al-Duri and Aichroth 2001** [10] | Retrospective study (Level IV) | 17 patients (18 knees) | Patellar tendinopathy | mean 12 months | - | - |
| **Owens et al 2001** [482] | Case series (Level IV) | 16 patients | recalcitrant lateral epicondylitis | mean 24.1 months | Reduction of pain at rest (pain score 0.58), pain with activities of daily living (pain score 1.58) and pain with sports and work (pain score 3.25); | No complications, including no nerve injury or instability |
| **Maquirriain et al 2002** [415] | Case series (Level IV) | 7 patients | chronic achilles tendinopathies | mean 16 months | Improvement of final outcome (rating system from 39 to 89) | Hematoma; edema |
| **Budoff et al 2005** [89] | Case series (Level IV) | 60 patients (62 shoulders) | rotator cuff | 114 months | Improvement of function (50% of excellent results with UCLA shoulder score); reduction of pain (no pain in 58% of patients operated) | Decreased passive range of motion |
| **Cummins et al 2006** [142] | Case series (Level IV) | 18 patients | chronic lateral epicondylitis | Mean 21.6 months | Reduction of worst level of pain (VAS from 8.6 to2.2), pain at rest (VAS from 4.3 to 0.3), pain lifting a heavy object (VAS from 8.2 to 1.4), pain with repetitive lifting (VAS from 7.5 to 1.6) and night pain (VAS from 5.6 to 0.5) | No complications |
| **Ogon et al 2006** [469] | Prospective study (Level IV) | 15 patients | chronic patellar tendinopathy | mean 41 months | Improvement of function (Blazina score from 3.7 to 0.4); reduction of tendon edema | - |
| **Jerosch and Schunck 2006** [286] | Prospective study (Level IV) | 20 patients | lateral epicondylitis | mean 21 month | Reduction of subjective pain at rest (VAS from 5.0 to 0.5), pain at daily living activities (VAS from 6.0 to 1.0) and pain at athletic activities (VAS from 7.3 to 1.2); improvement of function (from 5.2 to 10.9) | Local synovitis; presence of plica humeroradialis as additional alterations. No postoperative instability or other complications. |
| **Willberg et al 2007** [674] | Prospective study (Level IV) | 15 patients | Jumper's knee-patellar tendinopathy | mean 13 month | Reduction of pain during their actual sport activity (VAS from 79 to 12); | - |

**Table 10** (*continued*)

| Study (references) | Level of evidence | N° patients | Tendon | Follow up | Outcome | Complications |
|---|---|---|---|---|---|---|
| **Lorbach et al 2008** [379] | Case series (Level IV) | 20 patients | chronic patellar tendinopathy | 24 months | Improvement of performance and activity level (Tegner score from 4.4 to 7.95; Lysholm score from 57.1 to 97.3); reduction of pain related to functioning and activities (Kujala score from 53.7 to 95.4); | No postoperative complications (wound infections or revisions) |
| **Vega et al 2008** [654] | Prospective study (Level IV) | 8 patients | chronic Achilles tendinopathy | Mean 27.1 months | Excellent clinical outcome (Nelen scale); disappearance or considerable decrease in nodular swelling; decrease in thickening of the tendon | No signs of flexion-extension deficit; no pain or cosmetic problems with the resulting scars. |
| **Baker and Baker 2008** [49] | Case series (Level IV) | 40 patients (42 elbows) | recalcitrant lateral epicondylitis | mean 130 months | Reduction of pain (pain score at rest 0, pain score with ADLs 1.0, pain score with work or sports 1.9); improvement of function (functional subscore of the MCEPI 11.7 out of 12 points) | - |
| **Grewal et al 2009** [237] | Prospective study (Level IV) | 36 patients with chronic lateral epicondylitis with an arthroscopic release | chronic lateral epicondylitis | Mean 42 months | Reduction of pain (PRTEE for pain 14.6 out of 50, ASES-e pain score 16.1, where 0=no pain); improvement of function (PRTEE for functional disability 11.3 out of 50, ASES-e function score 27.9, where 36=full function); global improvement (total PRTEE 26.2 out of 100, Mayo Elbow Performance Index (MEPI) score 78.6) | - |
| **Wada et al 2009** [659] | Prospective study (Level IV) | 18 patients (20 elbows) | Chronic lateral epicondylitis | 28 months | Reduction of pain at rest (VAS from 3.9 to 0.3), pain during activity (VAS from 7.8 to 0.9); improvement of function (JOA elbow score from 29.2 to 89.9) | No complications, including nerve injury or instability |

## III. Best modalities for management of tendinopathy

In general, it would be reasonable to treat a patient with tendinopathy with physical therapy involving a programme of eccentric exercises, to be performed for 12 weeks. If the condition does not respond to this intervention, shock wave therapy, or nitric oxide patch might be considered, although data on their efficacy are limited. If the condition does not respond to these interventions, injections could be considered. The use of operative treatment should be discussed with the patient after at least three to 6 months of non-operative management. Moreover, patients should understand that symptoms may recur with either conservative or operative approaches.

## IV. The future and conclusions

In the last few decades, biomaterials have become critical components in the development of effective new medical therapies for wound care [40, 133]. Many new tissue engineered materials have been introduced, including artificial polymers, biodegradable films and biomaterials derived from animal or human tissues.

Biological scaffolds are protein-based extracellular matrices which usually derive from human or animal connective tissues [114]. Advantages of biological scaffolds include a well-defined three dimensional microstructure (allowing host cell integration) and natural porosity (which provide much larger space for host cell attachment, proliferation, migration and assists gas and metabolite diffusion). These proprieties allow biological scaffolds to quickly interact with host tissue and induce new tissue formation faster than synthetic scaffolds. Limitations of biological scaffolds are their poor mechanical properties, undefined rate of degradation, variation in biocompatibility, propensity to induce an inflammatory response and potential for implant rejection [114].

On the other hand, synthetic scaffolds are manufactured from chemical compounds [114], which permit better control of the chemical and physical properties leading to stronger mechanical strength and consistency in quality. However, biocompatibility of synthetic scaffolds is very poor, as they can never be absorbed or integrated into host tissue. High incidences of postoperative infection, and chronic immune response have been reported with the use of such materials [114].

A genetic component has been implicated in tendinopathies, but investigations into the genetic factors involved in their etiology are still in their infancy [350]. An enhanced understanding of these factors holds the promise of new approaches to the prevention and management of these common conditions. Further randomized controlled trials are necessary to better clarify the best therapeutic options for the management of tendinopathy.

# Pathogenesis of rotator cuff tears

# Chapter 5

## Histopathology of the supraspinatus tendon in rotator cuff tears

## ABSTRACT

**Background:** Causes of rotator cuff pathology are poorly understood.

**Hypothesis:** Macroscopically intact supraspinatus tendon may show profound light microscopy changes.

**Study Design:** Comparative laboratory study.

**Methods:** Tendon samples were harvested from 88 individuals (49 men, 39 women; mean age: 58.2 years) who underwent arthroscopic repair of a rotator cuff tear, and from 5 male patients who died of cardiovascular events (mean age: 69.6 years). A full thickness supraspinatus tendon biopsy was harvested *en bloc* within the arthroscopically intact middle portion of the tendon. Using Haematoxylin and Eosin staining, slides were assessed twice by the same examiner using a semiquantitative grading scale assessing fiber structure and arrangement, rounding of the nuclei, regional variations in cellularity, increased vascularity, decreased collagen stainability and hyalinization. Intra-observer reliability of the subscore readings was calculated.

**Results:** The mean pathologic sum-score of ruptured tendons was significantly greater than the mean pathologic score of control tendons. Within each specific category of tendon abnormalities, the control and ruptured tendons were significantly different (chisquare test); all variables were significantly different. There was good agreement between the two readings.

**Conclusions:** Nonruptured supraspinatus tendons, even at an advanced age, and ruptured supraspinatus tendons are clearly part of two distinct populations.

**Clinical Relevance:** During cuff repair, it is not necessary to excessively freshen the torn tendon to bleeding tissue: the macroscopically intact supraspinatus tendon is degenerated as well, and the failed healing response is not limited to the ends of the torn tendon.

## INTRODUCTION

Rotator cuff pathology is frequent, and produces a marked impact on health costs in industrialized countries, being the most frequently encountered cause of pain and dysfunction in the shoulder. The aetiology of rotator cuff pathology is still debated, and the natural history of rotator cuff tendinopathy is still unclear. Probably, rotator cuff tendinopathy is secondary to multiple factors [202, 423, 429].

Combinations of intrinsic and extrinsic factors play vital roles in the development of injury to the rotator cuff. Intrinsic factors focus on direct injury to the rotator cuff via tensile overload, aging, or microvascular supply through traumatic, reactive, or degenerative insults to the rotator cuff [20]. Extrinsic factors induce injury to the rotator cuff through compression of the tendons by bony impingement or direct pressure from the surrounding soft tissue [20, 423].

Some authors have attempted to correlate the incidence of rotator cuff tears with the compression of the tendons by direct pressure from surrounding soft tissue or bony impingement [69, 459]. On the other hand, many authors suggested that pathological changes in the supraspinatus tendon are the cause of the weakening that is responsible for rotator cuff lesions, triggered by microtrauma [468, 483]. Pathological changes of tendons can lead to reduced tensile strength and a predisposition to rupture [33, 251].

In favor of the intrinsic theory, in rotator cuff pathology partial lesions occur more often on the articular side of the tendon [202, 203, 216]. Matthews et al [423] reported on the morphological changes in the macroscopically intact portion of a torn supraspinatus tendon, but their controls came from patients with glenohumeral instability, approximately 40 years younger than their patients with rotator cuff tears.

In the present study, we analyze the histopathological features of surgical specimens of supraspinatus tendon from patients with rotator cuff tears. We hypothesised that the macroscopically intact supraspinatus tendon shows changes that may be shown by microscopic examination, and can represent the pathogenic precursor to a subsequent rotator cuff tear. A secondary aim of the study was to evaluate the reliability of histopathologic evaluation of tendon tissue in rotator cuff pathology, as already demonstrated in the Achilles [390] and patellar tendon [401].

## MATERIALS AND METHODS

All procedures described in this study were approved by the Ethics Committee of our University. All patients gave written informed consent.

## Tendon Samples

*Ruptured rotator cuff tendons (N = 88 Tendons)*

Samples from the macroscopic intact portion of ruptured supraspinatus tendons were obtained from patients (49 men, 39 women; mean age, 58.2 years) who had sustained a rotator cuff tear and underwent arthroscopic repair of the lesion at our centre in the period January 2004 to September 2006.

Conservative management, including nonsteroidal anti-inflammatory drugs, physiotherapy and rest, failed in all patients, and they continued to experience unacceptable pain and weakness in the affected shoulder. None of the patients had received injections of corticosteroids and none had undergone prior surgery on the affected shoulder. All patients fulfilled the following criteria: (1) positive rotator cuff lag signs on pre-operative examination (at least one among Jobe test, Napoleon test, lift-off test, and Patte test) [532] (2) no episodes of shoulder instability, (3) no radiographic sign of fracture of the glenoid or the tuberosities, (4) Magnetic resonance imaging (MRI) evidence of cuff tear, (5) rotator cuff tear of 1 or more tendons at arthroscopic examination, (6) no lesion of the glenoid labrum or of the capsule at arthroscopic examination.

The rotator cuff tears were classified as small (< 1 cm) in 11 patients, medium (1 to 3 cm) in 19 patients, large (3 to 5 cm) in 30 patients and massive (> 5 cm) in 28 patients.

At arthroscopy, a full thickness supraspinatus tendon biopsy about 4 x 4 mm in size was harvested *en bloc* within the arthroscopically intact middle portion of the tendon between the lateral edge of the tendon tear and the muscle-tendon junction.

*Nonruptured Rotator Cuff Tendons from Deceased Patients (N = 10 Tendons)*

The right and left supraspinatus tendons were obtained from each of 5 male patients who died of cardiovascular events (mean age, 69.6 years). The tendons were harvested in the *post mortem* room under sterile conditions. The rotator cuff tendon was freed from surrounding tissue and as much muscle and fat as possible were removed. From questioning the patients' relatives and from consultation of the hospital notes, it was learned that no patient had sustained an acute or overuse injury to the rotator cuff tendon, and no patient had taken corticosteroids during the past 5 years.

## Staining Procedures

All the tendon samples were placed in 20 ml of sterile 10% formalin in a universal container for transportation to the Pathology Department. Once fixed with buffered 10% formalin, the pieces were dehydrated, embedded in paraffin and cut at 4 µm sections. Finally, sections were stained with Haematoxylin and Eosin, and were examined both under white light as well as under polarized light microscopy.

### Assessment of Tendon Lesions

For each tendon and each staining technique, three slides were randomly selected and examined using a light microscope. The identification number on each slide was covered with a removable sticker, and each slide was numbered using randomly generated numbers. After one of the authors interpreted all the slides once, the stickers were removed, a new sticker was applied, and the slides were renumbered using a new series of randomly generated numbers. The degree of staining of all the slides was reassessed by the same author, and the two results were compared.

If an inconsistency (more than one grade on the scoring system described in Table 2) existed between the two results, the slides were reassessed with the help of a Consultant Pathologist (CR) who has a special interest in musculoskeletal abnormalities.

The area of each specimen showing the most advanced pathologic changes was selected, and the worst possible results for each slide were used in this study.

The slides were interpreted using the modified semi-quantitative grading scale [36, 131, 390, 391, 401, 451, 621] which assesses various aspects of tendon tissue. The variables included in the scale are 1) fiber structure, 2) fiber arrangement, 3) rounding of the nuclei, 4) regional variations in cellularity, 5) increased vascularity, 6) decreased collagen stainability, and 7) hyalinisation.

A four-point scoring system was used, where 0 indicates a normal appearance, 1 indicates a slightly abnormal appearance, 2 a moderately abnormal appearance, and 3 a markedly abnormal appearance. Overall, the total score for a given slide could vary between 0 (normal tendon) and 21 (most abnormal appearance detectable).

### Statistics

Kappa statistics were used to assess the agreement between the scoring of the slides. The chi square test was used to ascertain the association between the type of tendon (control or ruptured) and the pathologic score. Because the pathologic scores were not normally distributed, the Mann-Whitney U-test was used to determine whether the sum-score difference between the two tendon groups was statistically significant. A probability level of $< 0.05$ was considered significant.

## RESULTS

The mean pathologic sum-score of ruptured tendons was greater than the mean pathologic score of control tendons (15.66 ± 1.82 versus 3.7 ± 2.31, $P = 0.001$) (Table 1). Within each specific category of tendon abnormalities, the chisquare test showed significant

difference between the control and ruptured tendons; all the variables were significantly different ($P < 0.05$).

**Table 1:** Summary of Pathologic Scores of Control and Ruptured Tendons. The worst scoring result was used for each situation.

| Total tendon pathologic score | Control tendons | Ruptured tendons |
|---|---|---|
| **Mean** | 3.7 | 15.66 |
| **Median** | 2.5 | 16 |
| **SD** | 2.3 | 1.82 |
| **Range** | 1-7 | 10-21 |

The distribution of the scores for each category marked is shown in Table 2. Using the kappa statistics, the agreement between the two readings ranged from 0.56 to 0.86 (Table 3).

**Table 2:** Distribution of Tendon Pathologic Scores. The worst scoring result was used in each instance.

| Variable | Control tendons (N = 10) | | | | Ruptured tendons (N = 88) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| **Fiber structure** | 6 | 3 | 1 | 0 | 0 | 8 | 32 | 48 |
| **Fiber arrangement** | 6 | 2 | 2 | 0 | 0 | 11 | 31 | 46 |
| **Rounding of the nuclei** | 5 | 4 | 1 | 0 | 0 | 1 | 36 | 51 |
| **Regional variations in cellularity** | 6 | 2 | 2 | 0 | 0 | 0 | 35 | 53 |
| **Increased vascularity** | 6 | 3 | 1 | 0 | 0 | 0 | 35 | 53 |
| **Decreased collagen stainability** | 7 | 2 | 1 | 0 | 0 | 13 | 24 | 51 |
| **Hyalinization** | 6 | 3 | 1 | 0 | 54 | 20 | 10 | 4 |

**Table 3:** Kappa Scores for Each Variable 1 indicates a perfect match, and 0 represents no match.FS: fiber structure; FA: fiber arrangement; N: rounding of the nuclei; RVC: regional variations in cellularity; V: increased vascularity; DCS: decreased collagen stainability; H: hyalinization.

| Tendons | Kappa value | | | | | | |
|---|---|---|---|---|---|---|---|
| | FS | FA | N | RVC | V | DCS | H |
| **All** | 0.76 | 0.72 | 0.81 | 0.77 | 0.73 | 0.69 | 0.62 |
| **Ruptured** | 0.62 | 0.63 | 0.85 | 0.82 | 0.79 | 0.86 | 0.66 |
| **Control** | 0.82 | 0.78 | 0.75 | 0.74 | 0.61 | 0.56 | 0.58 |

## Assessment of Each Variable

*Fiber Structure*. In the control specimens, the fibers were arranged close and parallel to each other with slight waviness (Figure 1). Increased waviness and separation of the fibers accompany slight and moderate changes. Markedly abnormal specimens showed loss of the finer fiber structure (Figure 2). The median pathologic score for the control tendons was 0, compared with 3 for the ruptured tendons.

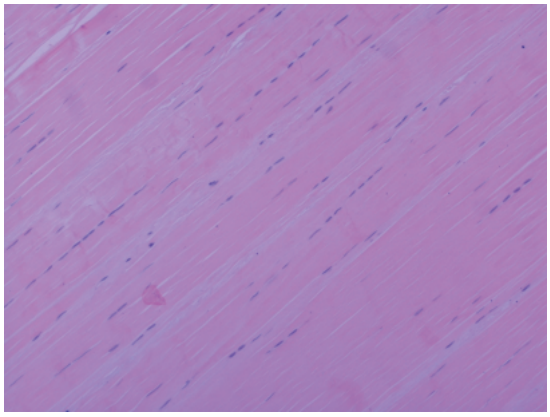

**Figure 1:** Hematoxylin and eosin stain of a control supraspinatus tendon in a 71-year-old man. Note the closely packed, lightly stained parallel bundles of collagen fibers that contain flattened nuclei of tenocytes. (Original magnification: x150). Fiber structure: 0. Fiber arrangement: 0. Rounding of the nuclei: 0. Regional variations in cellularity: 1. Increased vascularity: 0. Decreased collagen stainability: 0. Hyalinisation: 0.

*Fiber Arrangement*. In the control tendons, the fibers were arranged parallel to each other. In ruptured and tendinopathic samples, this parallel arrangement was lost and haphazard. The median for the control tendons was 0, and for the ruptured tendons it was 3.

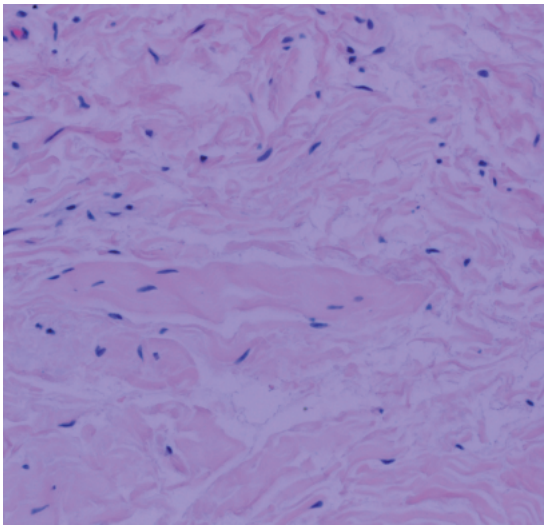

**Figure 2:** Hematoxylin and eosin stain of supraspinatus tendon harvested from the intact middle portion of the tendon between the lateral edge of the tendon tear and the muscle-tendon junction in a 53-year-old man. The collagen fibers have an undulating distribution, and the whole area is hypercellular. (Original magnification: x150). Fiber structure: 2. Fiber arrangement: 2. Rounding of the nuclei: 1. Regional variations in cellularity: 1. Increased vascularity: 1. Decreased collagen stainability: 2. Hyalinisation: 0.

*Tenocyte Nuclei*. Normally, the tenocyte nuclei were flattened and spindle shaped, sometimes arranged in rows (Figure 1). In the ruptured and tendinopathic samples, the tenocytes first decreased in number; then, as the pathologic changes progressed, the

nuclei became progressively rounded. In some instances, these tenocytes resembled chondrocytes (Figure 3). The median for the control tendons was 0.5, and for the ruptured tendons it was 3.

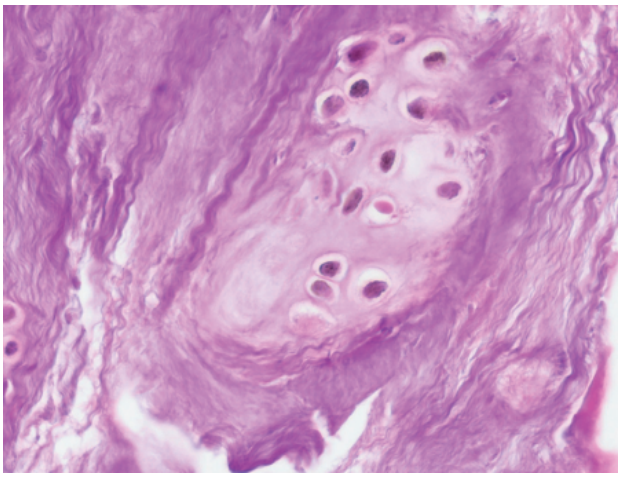

**Figure 3:** Hematoxylin and eosin stain of supraspinatus tendon harvested from the intact middle portion of the tendon between the lateral edge of the tendon tear and the muscle-tendon junction in a 62-year-old woman. Abnormal tenocytes resembling chondrocytes are shown. (Original magnification: x150). Fiber structure: 2. Fiber arrangement: 2. Rounding of the nuclei: 3. Regional variations in cellularity: 2. Increased vascularity: 0. Decreased collagen stainability: 1. Hyalinisation: 0.

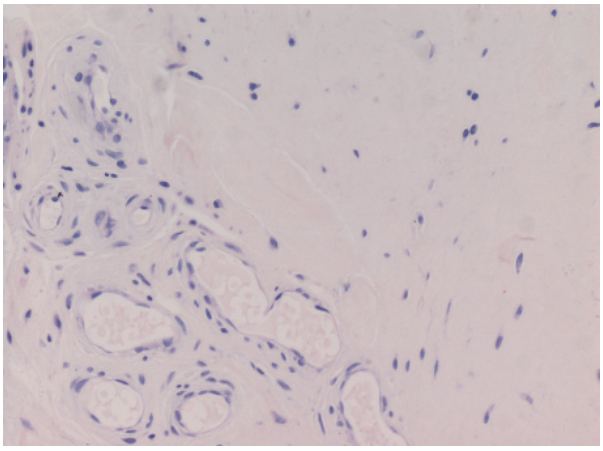

**Figure 4:** Hematoxylin and eosin stain of supraspinatus tendon harvested from the intact middle portion of the tendon between the lateral edge of the tendon tear and the muscle-tendon junction in a 59-year-old man showing abnormal neovessels. (Original magnification: x200). Fiber structure: 2. Fiber arrangement: 2. Rounding of the nuclei: 1. Regional variations in cellularity: 2. Increased vascularity: 3. Decreased collagen stainability: 2. Hyalinisation: 0.

*Cellularity*. The whole slide was assessed for areas of increased cellularity. The median for the control tendons was 0, and for the ruptured tendons was 3.

*Vascularity*. Vascular bundles usually run parallel alongside the collagen fibers. The number of these vascular bundles increases with degeneration of the tendon (Figure 4). The median for the control tendons was 0, and for the ruptured tendons it was 3.

*Collagen Stainability*. Normal collagen colors a deep pink-red when hematoxylin and eosin stain is added. However, with degenerated collagen, the section stainability is reduced and appears paler. This pallor was graded. The median value for the control tenons was 0, and for the ruptured tendons it was 3.
*Hyalinization*. Very few specimens showed any evidence of hyalinization, and analytical statistics showed that this histopathological criterion was poorly reproducible.

## DISCUSSION

The supraspinatus tendons of patients undergoing arthroscopic repair for a rupture show profound histopathologic changes, while the tendons of aged persons with no known tendon abnormalities have, as a group, little histologic evidence of pathological changes. Moreover, tendon changes are not only localized at the site of rupture, but also in the macroscopic intact tendon portion.

### Histopathology of Ruptured and Aging Rotator Cuff Tendons

The histopathologic appearance of our rotator cuff tendon rupture specimens demonstrated a condition of tendinous pathology similar to described by previous authors [202, 203]. Ruptured supraspinatus tendons show marked collagen degeneration and disordered arrangement of collagen fibers [202, 203].

In the study by Matthews et al [423], the patients in control group had glenohumeral instability and were approximately 40 years younger than the patients with rotator cuff tears. Our control patients, on the other hand, did not have a past medical history of shoulder problems, and were nearly 20 years older than our patients with rotator cuff tears.

Many studies have attempted to correlate the incidence of rotator cuff tears with the compression of the tendons by direct pressure from surrounding soft tissue or bony impingement. In 1972, Neer [459] proposed that the majority of rotator cuff tears result from mechanical compression of the tendons under the coracoacromial arch. Successively, Bigliani [69] reported a correlation between acromial morphology and rotator cuff tears, showing that the Type III acromion was present in the majority of rotator cuff tears. Many authors [202, 203] showed that pathologic changes can occur at the bursal side of the rotator cuff, suggesting a role of friction and rubbing played from the undersurface of the acromion.

On the other hand, various authors advocated that intrinsic factors instituted rotator cuff pathology [555, 556]. Specifically, the proposed causes of intrinsic degeneration are aging, tensile overload and microvascular supply.

Codman [124] advocated pathological changes of the tendon as the primary cause of rotator cuff lesions, and showed that the initiating pathological change appeared to be a peeling back of the articular margin of the insertion of the supraspinatus tendon, a feature that he termed the 'rim-rent lesion'. Ozaki et al [483] confirmed these findings in a histological study of block dissections of the humeral head, rotator cuff and acromion, demonstrating how macroscopic and microscopic tearing to the articular margin of the rotator cuff insertion precede histological changes in the acromion, suggesting an intrinsic cause.

Sano et al [555] in a cadaveric study found more pronounced pathological changes in the articular than in the bursal portion of the rotator cuff, and proposed that intrinsic degeneration might constitute the primary cause of rotator cuff lesions.

Hashimoto et al [251] showed that pathological changes of the tendon pre-date the rupture. These authors analyzed 80 medial stumps of rotator cuff tendons obtained along the torn edge of each stump. Instead, in this study we harvested the tendon specimens in the middle portion of the macroscopic intact supraspinatus tendon, between the muscle-tendon junction and the tendinous humeral insertion. Furthermore, Hashimoto et al had no controls.

Our supraspinatus tendon sample showed an increase in the number of tenocytes with rounded nuclei. The histologic appearance was of poor healing response with absence of acute inflammation.

Aging may result in functional and structural changes in human tendons, with an increase in total collagen content and collagen fiber diameter and a decrease in collagen turnover. The increase in collagen fiber diameter is probably due to several smaller fibrils becoming mechanically coupled so they can transmit mechanical stresses in concert. However, there is little proof that tendons from healthy, older persons exhibit histologic evidence of degeneration, and this is confirmed by the results of the present investigation. The changes in both cellular and fibrous components, with decrease in the average maximum diameter and density of collagen fibrils and an increase of fibril concentration, are most likely related to the decreased functional requirements. In healthy animals, the mechanical properties of tendons remain constant after the end of growth well into senescence [390].

**Reliability of Histopathologic Interpretation**

In the present study, each slide was scored twice with the help of a consultant pathologist who has a special interest in the musculoskeletal system. Despite specific training, the agreement of blinded assessment for the various components of the scoring system is, at best, acceptable (Table 3). This underlines how difficult it can be to recognize specific

patterns in tendon abnormalities, and the importance of having well-trained individuals to interpret the slides, especially if only a limited number of histologic techniques are used. To improve the reproducibility of these readings, the assessment would have to be performed several times, with the slides being randomly reordered each time. Also, large populations of samples or other methods of assessing performance, possibly with weighted outcomes, would be required. Finally, two or more researchers scoring the tendons would decrease observer bias. Whether these methods could be implemented in clinical practice or in research studies is open to discussion.

In concert with previous investigations [36, 131, 390, 391, 401, 451, 621], we used a semi-quantitative assessment of the tendinopathic lesions observed. We are conscious of the limitations of this assessment system, as we categorise in four classes (from 0, i.e. fully normal, to 3, i.e. markedly abnormal) a qualitative evaluation of several aspects of the histopathological appearance of the tendon section examined. It is desirable that the fully automated image analyses systems used in other fields of musculo-skeletal medicine will be used in this field as well, and thus allow a more objective quantification of the abnormal appearance of tendinopathic tendons.

## Clinical Implications

A clinically relevant finding is that, for each variable and for the tendons as a group, the control and the ruptured tendon groups were significantly different (chi-square and Mann-Whitney *U*-test, $P < 0.001$). It would be expected that at least some of the difference between the tendon scores could be accounted for by the age difference between the samples. However, an older tendon is more likely to have an impaired blood supply and greater age-induced pathological changes than tendons from younger persons. The control tendons in this study came from a slightly older group of patients. Nevertheless, the tendons from persons aged approximately 70, who had no previously known tendon ailments, were significantly less degenerated than the tendons of patients aged 60 who had rotator cuff ruptures. It is therefore conceivable that significant mechanical stresses must have acted on already altered tissue to exceed its tensile resistance and induce a tear.

In our study, tendon changes were not only localized at the site of rupture, but occur also in the middle portion of the macroscopic intact supraspinatus tendon. We can speculate that it is not necessary during arthroscopic cuff repair to excessively freshen the torn tendon to bleeding tissue, because, in the presence of a rotator cuff tear, the macroscopically intact supraspinatus tendon is degenerated as well, and the failed healing response is not limited to the ends of the torn tendon. Therefore, the tissue at the distal end of the tendon may be left intact at the time of repair. Probably, the tendon itself does not contribute to healing [646], and a limited freshening of the frayed ends is sufficient, saving more tendon tissue, preventing excessive tension of the repair.

### Limitations of the Present Study

We are fully aware of the limitations of this study. For example, the tendons that we considered normal came from patients with various degrees of vascular disease. However, the rotator cuff tendon is normally a relatively avascular structure, and it is thus likely that our tendon samples were representative of normality, given the age of the patients. The ideal control should not have shoulder pathologies. However, for ethical and practical reasons, no alternatives were possible, as it is impossible in our setting to take surgical biopsies from healthy individuals. Furthermore, we believe that the differences between the control and ruptured supraspinatus tendons are strong enough to justify our conclusions.

Also, as aging causes at least some morphologic changes in the tendons, and given that our control tendons were harvested from donors older than patients with a torn supraspinatus tendon, the use of an age-matched control population would have further highlighted the histologic differences that we have described. When interpreting the results of this study, it should be pointed out that we used only one staining method (hematoxylin and eosin). Obviously, the fact that more advanced histochemical and immunohistochemical techniques and electron microscopy— to detect, for example, extra lipids, calcium deposits, collagen denaturation, pathologic tenocyte metabolism, collagen types, and foreign materials—were not used may have resulted in an underestimation of tendon abnormalities in the control group. However, the staining employed in the present study is widely available, is cost-effective, and requires little technical ability. Also, most pathologists are familiar with hematoxylin and eosin staining, and are used to interpreting a variety of specimens stained in this fashion. Finally, we are not aware of the level of rotator cuff tendon degeneration in the general Italian adult population. We are not aware of any study detailing the histologic appearance of rotator cuff tendon degeneration in this population.

## CONCLUSIONS

Unruptured rotator cuff tendons, even at an advanced age, and ruptured supraspinatus tendons are clearly part of two distinct populations. In ruptured rotator cuff tendon, the collagen distribution is abnormal [423]. Tenocytes from ruptured tendons produce greater quantities of type III collagen than tenocytes from normal tendons [391]. This altered production of collagen may be one reason for the histologic alterations described in this study, and may result in the tendon being less resistant to tensile forces, and thus at increased risk of rupture.

# Chapter 6

## Light microscopic histology of supraspinatus tendon ruptures

## ABSTRACT

We analyzed the morphological features of the human surgical specimens of supraspinatus tendon from patients with rotator cuff tears. Tendon samples were harvested from 31 subjects (21 men and 10 women; mean age 51 years, range, 38 to 64) who underwent arthroscopic repair of a rotator cuff tear, and from 5 male patients who died of cardiovascular events (mean age, 69.6 years). Histologic examination was performed using Haematoxylin and Eosin, Masson´s Trichrome, and Van Gieson's connective tissue stain. The specimens were examined twice by the same examiner under white light and polarized light microscopy. Particular effort was made to asses any evidence of the changes associated with tendinopathy. Within each specific category of tendon abnormalities, the chi square test showed significant differences between the control and ruptured tendons ($P < 0.05$). Using the kappa statistics, the agreement between the two readings ranged from 0.57 to 0.84. We found thinning and disorientation of collagen fibers and chondroid metaplasia to be more pronounced on the articular side of the specimens from patients with rotator cuff tear ($P < 0.05$). The present study provides a description of the histological architecture of human surgical specimens of normal supraspinatus tendon from patients with rotator cuff tears, and demonstrates more frequent tendon changes on the articular side of the rotator cuff.

## INTRODUCTION

Rotator cuff pathology is frequent, and causes great healthcare costs in industrialized countries. Over the last decade, novel therapeutic strategies have been developed for rotator cuff tears. While many of the epidemiological and imaging difficulties have been addressed, the aethiopathogenesis of rotator cuff disease remains poorly understood. Some studies have attempted to correlate the presence of a tear of the rotator cuff with the compression of the tendons by direct pressure from surrounding soft tissue or bony impingement [69, 138, 201, 459]. On the other hand, age-related degenerative changes in the supraspinatus tendon could be the cause of the weakening of the tendon which is responsible for rotator cuff lesions, triggered by microtrauma [124, 468, 483]. A failed healing response of the tendons could also lead to reduced tensile strength, and a predisposition to rupture [251].

Systemic histopathological studies examining pathological findings and their distribution in rotator cuff tendons are lacking in literature. We therefore undertook such a study of supraspinatus tendon samples obtained from patients undergoing arthroscopic repair of a rotator cuff tear to examine the distribution of tendinopathic changes associated with this condition.

## PATIENTS AND METHODS.

The study had the approval of the local ethics committee. Informed consent was obtained from each patient prior to surgery.

### Tendon Samples

*Ruptured rotator cuff tendons (N = 31 Tendons)*

Samples from ruptured supraspinatus tendons were obtained from patients (21 men and 10 women; mean age 51 years, range, 38 to 64) who had sustained a rotator cuff tear and underwent arthroscopic repair of the lesion at our centre. Conservative management, including nonsteroidal anti-inflammatory drugs, physiotherapy and rest, failed in all patients, and they continued to experience unacceptable pain and weakness in the affected shoulder. None of the patients had received injections of corticosteroids, and none had undergone prior surgery on the affected shoulder. All patients fulfilled the following criteria: (1) positive cuff signs on preoperative examination, (2) no episodes of shoulder instability, (3) no radiographic sign of fracture of the glenoid or the tuberosities, (4) Magnetic resonance imaging (MRI) evidence of cuff tear, (5) rotator cuff tear of 1 or more tendons at arthroscopic examination, (6) no lesion of the glenoid labrum or of the capsule at arthroscopic examination.

The dominant arm was affected in 14 patients. The average duration of symptoms before surgical intervention was 8.7 months (range, 3–37 months).

The rotator cuff tears were classified as small (< 1 cm) in 4 patients, medium (1 to 3 cm) in 9 patients, large (3 to 5 cm) in 16 patients and massive (> 5 cm) in 2 patients. The tear involved the supraspinatus tendon in 17 patients; the supraspinatus and infraspinatus tendons in 14 patients.

All patients had full-thickness rotator cuff tears. 16 patients had a Bigliani type I acromion morphology, 9 Bigliani type II, and 6 Bigliani type III.

At arthroscopy, a full thickness supraspinatus tendon biopsy was harvested close to the tear edge.

*Nonruptured Rotator Cuff Tendons from Deceased Patients (N = 10 Tendons)*

The right and left supraspinatus tendons were obtained from each of 5 male patients who died of cardiovascular events (mean age, 69.6 years). The tendons were harvested in the *post mortem* room under sterile conditions. The rotator cuff tendon was freed from surrounding tissue, and as much muscle and fat as possible were removed. From questioning the patients' relatives and from consultation of the hospital notes, it was learned that no patient had sustained an acute or overuse injury to the rotator cuff tendon, and no patient had taken corticosteroids during the past 5 years.

**Staining Procedures**

All the tendon samples were placed in 20 ml of sterile 10% formalin in a universal container for transportation to the Pathology Department. Once fixed with buffered 10% formalin, the pieces were cut into 2 layers (a superficial layer and a deep layer) in a plane parallel to the long axis of the tendon. Therefore, the area of the tendon was divided into two layers, the superficial and deep layers.

Then the sample were dehydrated, embedded in paraffin and cut at 4 µm sections. Finally, sections were stained with Haematoxylin and Eosin, Masson´s Trichrome, and Van Gieson's connective tissue stain and were examined both under white light as well as under polarized light microscopy.

**Assessment of Tendon Lesions**

For each layer and each staining technique, three slides were randomly selected and examined using a light microscope. The identification number on each slide was covered with a removable sticker, and each slide was numbered using randomly generated numbers. After one of the authors interpreted all the slides once, the stickers were removed, a new sticker was applied, and the slides were renumbered using a new series of randomly

generated numbers. The degree of staining was reassessed by the same author, and the two results were compared. If an inconsistency existed between the two results, the slides were reassessed with the help of a Consultant Pathologist (CR), who has a special interest in musculoskeletal abnormalities.

The area of each specimen showing the most advanced pathologic changes was selected, and the worst possible results for each slide were used in this study. The two layers, superficial and deep, were scored for tendinopathic changes.

Particular effort was made to asses any evidence of the changes associated with the process of failed healing response in tendons [18-23]. This included evidence of thinning and disorientation of collagen fibers, chondroid metaplasia, lipoid degeneration, and mucoid degeneration.

## STATISTICS

Kappa statistics were used to assess the agreement between the scoring of the slides. The chi square test was used to ascertain the association between the two groups of samples and the pathologic changes. A probability level of $P < 0.05$ was considered significant.

## RESULTS

Within each specific category of tendon abnormalities, the chisquare test showed association between the control and ruptured tendons; all the variables were significantly different ($P < 0.05$). Using the kappa statistics, the agreement between the two readings ranged from 0.57 to 0.84 (Table 1).

### Group 1 (Table 2)

**Table 1:** Kappa statistics scores for each variable. 1 indicates a perfect match, and 0 indicates no match. CF, Thinning and disorientation of collagen fibers; CM Chondroid metaplasia; LD, Lipoid degeneration; MD, Mucoid degeneration

| Tendons | Kappa value | | | |
|---|---|---|---|---|
| | CF | CM | LD | MD |
| **All** | 0.63 | 0.77 | 0.75 | 0.72 |
| **Ruptured** | 0.74 | 0.79 | 0.84 | 0.84 |
| **Control** | 0.57 | 0.73 | 0.71 | 0.65 |

**Table 2:** Distribution on tendon abnormalities in the superficial and deep layers of rotator cuff tears

| | Superficial layer | Deep layer |
|---|---|---|
| **Thinning and disorientation of collagen fibers** | **8** | **23** |
| **Chondroid metaplasia** | **2** | **18** |
| **Lipoid degeneration** | **7** | **5** |
| **Mucoid degeneration** | **9** | **6** |

*Thinning and Disorientation of Collagen Fibers*. In the superficial layer specimens, the fibers showed increased waviness and separation in 8 cases (Figure 1). In the deep layer specimens, increased waviness and separation of the fibers were present in 23 cases ($P < 0.05$).

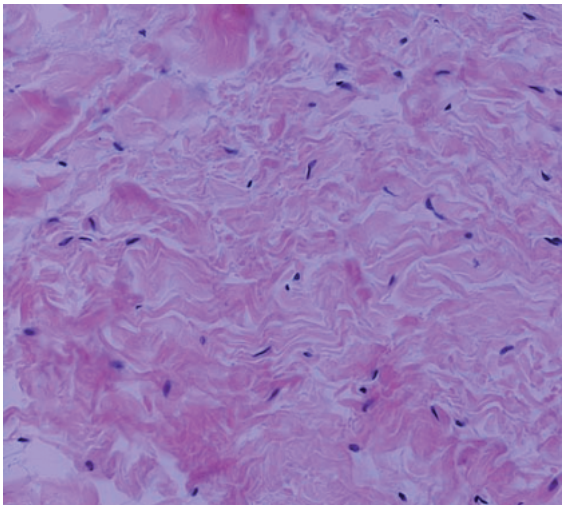

**Figure 1:** Surgical specimens of supraspinatus tendon from patients with rotator cuff tears displaying thinning and disorientation of collagen fibers.

*Chondroid metaplasia* In the superficial layer specimens, chondroid metaplasia (Figure 2) was present in 2 patients; in the deep layer specimens, 18 patients presented histological changes of chondroid metaplasia ($P < 0.05$).

*Lipoid degeneration.* Lipoid degeneration was present in 7 patients in the superficial layer specimens, and in 5 patients in the deep layer specimens ($P > 0.05$).
*Mucoid degeneration.* Mucoid degeneration was present in 9 patients in the superficial layer specimens, and in 6 patients in the deep layer specimens ($P > 0.05$).

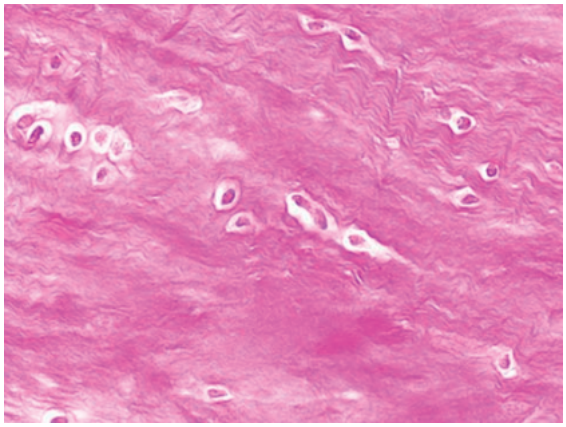

**Figure 2:** Van Gieson staining showed chondroid metaplasia

### Group 2

*Thinning and Disorientation of Collagen Fibers*. In the superficial layer specimens, the fibers showed increased waviness and separation in 1 patient. In the deep layer specimens, increased waviness and separation of the fibers were present in 1 patient.

*Chondroid metaplasia, Lipoid degeneration and Mucoid degeneration*. These changes were not present in any patient.

## DISCUSSION

The present study provides a description of the histological architecture of human surgical specimens of torn supraspinatus tendon from patients with rotator cuff tears, and shows that tendon changes occur more often on the articular side of the rotator cuff. The aetiology of rotator cuff tendinopathies and ruptures remains poorly understood. Rotator cuff tendinopathy has been attributed to a variety of intrinsic and extrinsic factors, which may have different roles in determining these lesions [20, 251].

Many authors [69, 138, 201, 459] have attempted to correlate the incidence of rotator cuff tears with the compression of the tendons by direct pressure from surrounding soft tissue or bony impingement. Extrinsic factors should produce injury to the rotator cuff by friction and rubbing, with consequent ultrastructural changes on the surface of the rotator cuff tendon [69, 138, 201, 459].

Neer [459] proposed that most rotator cuff tears result from mechanical compression of the tendons under the coracoacromial arch. Later, Bigliani [69] showed a correlation between acromial morphology and rotator cuff tears, as type III acromion was present in the majority of rotator cuff tears.

On the other hand, intrinsic degeneration and a failed healing response have been considered the primary cause of rotator cuff tears [124, 468, 483]. Tendon degeneration and a failed healing response would be linked to overuse, poor vascularity, lack of flexibility, genetic make-up, gender, endocrine or metabolic factors, with a reduced tensile strength and a predisposition to rupture [124, 468, 483]. Damage to the rotator cuff tendons can occur even if the cuff is stressed within its physiological limits, since frequent cumulative microtrauma may not leave enough time for the tendon involved repair [390, 391].

Codman [124] thought degenerative changes of the tendon the primary cause of rotator cuff lesions. Sano et al [555], in a cadaveric study, found more pronounced degenerative changes in the articular half than in the bursal half of the rotator cuff. These authors proposed that intrinsic degeneration might constitute the primary cause of rotator cuff lesions. Hashimoto et al [251] showed that degenerative changes of the tendon pre-exist before the rupture.

Budoff et al [88] proposed that the damaged supraspinatus tendon is unable to oppose the powerful pull of the deltoid muscle, with consequent inappropriate superior migration of the humeral head. The extrinsic impingement would be, therefore, caused secondarily by this superior migration.

We found most tendinopathic changes on the articular side of our specimens. The highest number of partial tears on the articular side probably results from the fact that this portion of the rotator cuff is exposed to higher stresses when compared with the bursal portion [382]. This would be in favour of primary intrinsic cause to the process of rotator cuff tear. If extrinsic impingement were the main cause of cuff degeneration, the highest incidence of tendinopathic changes would have been seen on the bursal side of the cuff.

In the study by Matthews et al [423], the patients in control group had glenohumeral instability and were approximately 40 years younger than the patients with rotator cuff tears. Our control patients, on the other hand, did not have a past medical history of shoulder problem, and were nearly 20 years older than our patients with rotator cuff tears.

The supraspinatus tendon is almost exclusively affected by insertional tendinopathy [646].

We found cartilage-like changes in patients affected by rotator cuff tears, but not in our control group. Recent biomechanical data suggest that the stress-shielded and transversely-compressed side of the enthesis has a distinct tendency to develop cartilage-like or atrophic changes in response to the lack of tensile load [393, 398, 399]. Over a long period, this process may develop a primary degenerative lesion in that area of the tendon. This may explain why the tendinopathy is not always clearly activity related, but more strongly correlated with age. In this manner, it could almost be considered an ‘‘underuse’’ injury rather than an overuse injury as a result of stress-shielding [393, 398, 399].

The formation of cartilage-like changes in the enthesis in many ways can be considered a physiological adaptation to the compressive loads [401, 404, 405]. It may not allow the tendon to maintain its ability to withstand high tensile loads in that region of the tendon. As

the stress-shielding may have led to tensile weakening over time, an injury may occur more easily in this region. In this manner, insertional tendinopathy could be considered an overuse injury, but predisposed by pre-existing weakening of the tendon [401, 404, 405].

Finally, we should consider the effects of differential strains within the rotator cuff tendon. As the joint changes position, strains in one section of the tendon could be changing in opposite directions. Internal shear forces and heat could be generated that injure cellular or matrix components in the tendon. Accumulation of these injuries could lead to significant tendon destruction.

In conclusion, thinning and disorientation of collagen fibers and chondroid metaplasia are more pronounced on the articular side of the specimens from patients with rotator cuff tear.The present study provides a detailed structural characterization of human surgical specimens of supraspinatus tendon from patients with rotator cuff tears, and may be useful for further investigations on the pathogenesis of rotator cuff tears.

# Chapter 7

## Higher fasting plasma glucose levels within the normoglycemic range and rotator cuff tears

## ABSTRACT

### Objective

To determine the plasma glucose levels in non diabetic patients with rotator cuff tear

### Design

Frequency-matched case-control study in a University Teaching Hospital

### Participants

The study included 194 subjects who were operated at our institution. Group 1 included 97 consecutive patients (36 men and 61 women; mean age: 62.9 years, range 37 to 82) who underwent arthroscopic repair of a rotator cuff tear in 2007 and 2008. Group 2 (control group) included 97 patients (36 men and 61 women; mean age: 61.6 years, range 36 to 80) who underwent arthroscopic meniscectomy for a meniscal tear in the same period, and had no evidence of shoulder pathology. These patients were frequency-matched by age (within 3 years) and gender with patients of Group 1.

### Results

Patients with rotator cuff tears (Group1) showed statistically significantly higher fasting plasma glucose levels within the normoglycemic range ($p = 0.007$) when compared with patients with meniscal tear (Group 2).

### Conclusions

The present study suggests that normal, but in the high range of normal, increasing plasma glucose levels may be a risk factor for rotator cuff tear. An enhanced understanding of these factors holds the promise of new approaches to the prevention and management of rotator cuff tears.

## INTRODUCTION

Tears of the rotator cuff are frequent, cause high health care costs in Western industrialized countries [429], and are the second most costly problem in Workers' Compensation systems, after low-back pain [255]. Over the last decade, novel diagnostic and therapeutic strategies have been developed. Although many of the imaging and surgical difficulties have been addressed, the mechanisms underlying the aethiopathogenesis of rotator cuff disease remains incompletely understood.

Both intrinsic and extrinsic theories of tendon injury have been proposed. Rotator cuff tears have been correlated to the compression of the tendons by direct pressure from surrounding soft tissue and bony impingement. On the other hand, age-related degenerative changes in the supraspinatus tendon could be the cause of the weakening of the tendon, which is responsible for rotator cuff lesions, triggered by microtrauma. Trauma to the shoulder is reported by up to 60% of patients, with the incidence being particularly high in overhead athletes (30%) and labourers (23%) [74]. Obesity and tobacco have been indicated as potential risks factors for rotator cuff tears [612, 670]. A genetic component has been implicated in tendon rupture and tendinopathies, but investigations into the genetic factors involved in the aetiology of tendinopathy are still in their infancy [409].

There is a possible relationship between hyperglycaemia and collagen structure alterations [526, 527]. At tissue level, tendons may be directly affected by non-enzymatic glycosylation processes which change collagen cross-links [48]. One of the underlying mechanisms of this cross-linking is the formation of advanced glycation endproducts [431].

The normal fasting plasma glucose level has been defined as less than 100 mg per decilitre (5.55 mmol per litre) [638]. Whether higher fasting plasma glucose levels within this range independently predict rotator cuff tear is unknown.

To our knowledge, no studies have focused on the correlation between plasma glucose levels and rotator cuff tears. We therefore undertook a frequency-matched case-control study of the plasma glucose level obtained from non diabetic patients undergoing arthroscopic rotator cuff repair, and compared them with a matched control group of patients of a similar age undergoing arthroscopic meniscectomy.

We wished to test the null hypothesis that there is no difference in plasma glucose level in patients presenting with an arthroscopically confirmed lesion of the rotator cuff and a control group.

## MATERIAL AND METHODS

The study included 194 subjects who were operated at our institution.

Group 1 included 97 consecutive patients (36 men and 61 women; mean age: 62.9 years, range 37 to 82) who underwent arthroscopic repair of a rotator cuff tear in 2007 and 2008.

Group 2 (control group) included 97 patients (36 men and 61 women; mean age: 61.6 years, range 36 to 80) who underwent arthroscopic meniscectomy for a meniscal tear in the same period, and had no evidence of shoulder pathology. These patients were frequency-matched by age (within 3 years) and gender with patients of Group 1. A match was obtained for all patients.

Patients in group 1 were included in the study if they had a rotator cuff tear diagnosed on clinical and imaging grounds and a rotator cuff tear found at the time of surgery. Patients of group 2 were included in the study if they had a meniscal tear diagnosed on clinical and imaging grounds and a meniscal tear found at the time of surgery. Patients were excluded from the study if they had primary osteoarthritis of the operated or contralateral joint, previous operations on the shoulder or knee, inflammatory joint disease, hypertension, diabetes, or hypercholesterolemia. Patients of the Group 2 were also excluded from the study if they had have history of shoulder pain, or rotator cuff pathology diagnosed by imaging or on clinical grounds.

### Anthropometric measurements

We measured height and weight of every patient on the day of the operation, and calculated the Body Mass Index (BMI). The same examiner measured all the subjects before blood sampling was performed.

### Measurement of plasma glucose levels

All blood samples were collected in an identical manner between 07.00 and 07.30. Patients fasted from midnight of the day before sampling. Biochemical analyses of blood were performed on fresh samples. Venous fasting plasma glucose levels were determined from blood samples collected in tubes containing sodium fluoride and delivered to the laboratory within two hours. Plasma glucose levels were determined using a BM/Hitachi 917 automated analyzer (Boehringer Mannheim). The analyser was calibrated weekly according to the manufacturer's instructions.

## STATISTICS

Data were entered in a commercially available database. Descriptive statistics were calculated, and analytical statistics were performed with non-paired sample *t*-test using Statistical Programs for the Social Sciences (SPSS). The correlation between BMI and glu-

cose concentration was analyzed in each group using Pearson's r. Significance was set at $P < 0.05$.

## RESULTS

The concentration of glucose **was** measurable in all patients.

Patients with rotator cuff tears (Group1) showed statistically significantly higher fasting plasma glucose levels within the normoglycemic range ($p = 0.007$) when compared with patients with meniscal tear (Group 2) (Table 1).

There was no difference in height, weight **or** BMI between the two groups (Table 2).

The positive correlation between BMI glucose concentration in both groups did not show evidence of a statistically significant association (group 1: $r = 0.2$, $p > 0.05$; group 2: $r = 0.2$, $p > 0.05$).

**Table 1:** Levels of plasma glucose (mg per decilitre) and millimoles per litre.

| Plasma glucose values | Group 1 (Patients with rotator cuff tears) | | Group 2 (Control group) | |
|---|---|---|---|---|
| | **mg per decilitre** | **millimoles per litre** | **mg per decilitre** | **millimoles per litre** |
| **Mean** | 99.17 | 5.5 | 95.45 | 5.3 |
| **Median** | 98 | 5.4 | 95 | 5.2 |
| **SD** | 9.04 | 0.5 | 9.87 | 0.55 |
| **Range** | 78-123 | 4.33-6.83 | 60-124 | 3.33-6.9 |

**Table 2:** Anthropometric measures (values in brackets are the range of values)

| | Group 1 (Patients with rotator cuff tears) | | Group 2 (Control group) | |
|---|---|---|---|---|
| **Gender** | Male=36 | Female=61 | Male=36 | Female=61 |
| **Height (cm)** | 1.70 (1.6-1.83) | 1.58 (1.48-1.75) | 1.74 (1.5-1.9) | 1.60 (1.5-1.8) |
| **Weight (kg)** | 80.97 (62-110) | 69.52 (48-107) | 81.05 (60-109) | 69.51 (48-96) |
| **BMI** | 27.90 | 27.81 | 26.97 | 26.85 |
| **Age** | Male=59.8 (37-73) | Female=64.75 (39-82) | Male=58.9 (36-73) | Female=63.2 (39-80) |

## DISCUSSION

This is the first study, to our knowledge, to examine plasma glucose levels in patients with rotator cuff tears. Patients with a rotator cuff tear had statistically significant higher fasting plasma glucose levels within the normoglycemic range than a control group with musculo-skeletal pathology of the lower limb.

The aetiology of rotator cuff tear rupture remains unclear. Combinations of intrinsic (age, gender) and extrinsic factors (such as load, sport and work) play a role in the devel-

opment of injury to the rotator cuff. Intrinsic factors focus on direct injury to the rotator cuff via tensile overload, aging, or microvascular supply through traumatic, reactive, or degenerative insults to the rotator cuff. Extrinsic factors induce injury to the rotator cuff through compression of the tendons by bony impingement or direct pressure from the surrounding soft tissue. Some authors have attempted to correlate the presence of rotator cuff tears with the compression of the tendons by direct pressure from surrounding soft tissue or bony impingement [20]. Other authors suggested that pathologic changes in the supraspinatus tendon are the cause of the weakening that is responsible for rotator cuff lesions, triggered by microtrauma. Pathologic changes of tendons can lead to reduced tensile strength and a predisposition to rupture.

Supporting the intrinsic theory, partial lesions in the rotator cuff occur more often on the articular side of the tendon. Siblings of patients diagnosed with full thickness tears of the rotator cuff had more than twice the relative risk for developing a lesion and nearly five times the risk of experiencing symptoms than spousal controls [249], implying a role for genetic factors [408]. There is an association between obesity and rotator cuff repair surgery in men and women aged 53 to 70, suggesting that increasing body-mass index is a risk factor for rotator cuff tendinopathies [670]. Theoretically, obesity may contribute to decreased vascularity through its associations with risk factors for vascular disease, such as elevated cholesterol [305], atherosclerosis [270], diabetes, hypertension, metabolic syndrome and decreased physical activity [592]. A correlation between adiposity and rotator cuff tendinopathy has been proposed, but, while the association with body-mass index and tendinopathy has been reported [670], no studies focused on plasma levels of glucose and rotator cuff tendinopathy

The essence of tendinopathy is a failed healing response, with haphazard proliferation and degeneration of tenocytes, disruption of collagen fibres, and subsequent increase in non-collagenous matrix. Accumulation of lipids and ground substance (glycosaminoglycans), and calcium deposits represent age-related changes of the tendon [297]. During ageing, lipid accumulation is extracellular: lipids with a high content of esterified cholesterol spread along the longitudinal axis of collagen fibres. Lipid deposition disrupts the fibre bundles, and thus decreases tendon strength [297]. At a tissue level, the tendon may be directly affected by non-enzymatic glycosylation processes which change collagen cross-links [48]. The biosynthesis of collagen is characterized by the presence of a large number of post-translational modifications such as hydroxylation and glycosylation of the polypeptide chains which are unique to collagen and a few other proteins [522]. The modification of collagen by glucose fixation on free amino groups of collagen is characterized by an altered solubility, and increased resistance to enzymatic digestion, and variations in crosslinking [522]. Since collagen is a widely distributed tissue protein, disturbance in its structure and function will have important consequences in many body organs [522]. The cross-linking of collagen by the non-enzymatic advanced glycation endproducts formation

or the enzymatic glucose incorporation has been indicated as one of the main mechanisms underlying the increased arterial stiffness in diabetic patients or diabetic complications in general [87].

Our data suggest a possible role of plasma glucose concentration in rotator cuff tear. Strengths of the present study include the systematic collection of blood samples, the use of pre-operative imaging and of arthroscopy to diagnose rotator cuff and meniscal tears, and the relatively large sample size of our study group. Nevertheless, we acknowledge the cross-sectional nature of the present investigation, which cannot completely resolve issues concerning temporality. The association between hyperglycaemia and the development of coronary heart disease is well established [576], and the management of diabetes is focused on reduction glucose levels [324]. We do not know whether such strategies might exert a beneficial effect on tendon problems as well. We are fully aware that more anthropometric measures could be performed (for example, waist and hip girth, and skinfold measurements), and this could be the subject of future endeavours.

In conclusion, there appears to be an association between plasma glucose level and rotator cuff tears. As this was a cross-sectional study, we could not determine temporality or rule out other factors that may influence rotator cuff tendinopathy. The present study suggests that increased, but in the high range of normal, plasma glucose levels may be a risk factor for rotator cuff tear. Additional research is required to improve our understanding of the association demonstrated in this study. An enhanced understanding of these factors holds the promise of new approaches to the prevention and management of these common conditions.

# Chapter 8

## Triglycerides and total serum cholesterol in rotator cuff tears: do they matter?

## ABSTRACT

### Objective

To determine the serum triglycerides and total serum cholesterol levels in patients with rotator cuff tear.

### Design

Frequency-matched case-control study in a University Teaching Hospital

### Participants

The study included 240 subjects who were operated at our institution. Group 1 included 120 patients (45 men and 75 women; mean age: 64.86 years, range 40 to 83) who underwent arthroscopic repair of a rotator cuff tear. Group 2 (control group) included 120 patients (45 men and 75 women; mean age: 63.91 years, range 38 to 78) who underwent arthroscopic meniscectomy for a meniscal tear, and had no evidence of shoulder pathology. These patients were frequency-matched by age (within 3 years) and gender with patients of Group 1.

### Main outcome measure

Measurement of serum triglyceride and total cholesterol concentrations.

### Results

When comparing the two groups, there was no difference either in serum triglyceride concentration or total serum cholesterol concentration.

### Conclusions

There appears to not be an association between serum triglyceride concentration and total serum cholesterol concentration and rotator cuff tears.

## INTRODUCTION

Rotator cuff pathology is a very common orthopaedic problem, and it is a cause of great healthcare costs in industrialized countries [172, 429]. Despite the relevance of the problem, the aetiology and pathogenesis of rotator cuff pathology remains unclear. Several theories of tendon injury have been proposed, and the incidence of rotator cuff tears increases with advancing age [339, 340, 423, 530, 531]. Trauma to the shoulder is reported by up to 60% of patients, and the incidence is particularly high in overhead athletes (30%) and labourers (23%) [74]. Obesity [670] and increased plasma glucose levels have been indicated as potential risk factor for rotator cuff tears. There are data on the possible relationship between high serum lipid concentration and complete rupture of the Achilles tendon [417, 485]. However, to our knowledge, no studies have focused on the correlation between serum lipid levels and rotator cuff tears.

We therefore undertook a cross-sectional study of the serum triglyceride concentration and total serum cholesterol concentration in patients undergoing arthroscopic rotator cuff repair, and compared them with a control group of patients of a similar age undergoing arthroscopic meniscectomy.

## MATERIAL AND METHODS

All procedures described in this study were approved by the Ethics Committee of our Institution. All patients provided written informed consent according to the Declaration of Helsinki.

The study included 240 subjects who were operated on at our institution. 140 participants (70 in the study group and 70 in the control group) from a prior investigation were included in this material, along with a further 100 subjects.

Group 1 included 120 patients (45 men and 75 women; mean age: 64.86 years, range 40 to 83) (Table 1) who underwent arthroscopic repair of a rotator cuff tear. The dominant arm was affected in 83 patients. The rotator cuff tears were classified as small (< 1 cm) in 15 patients, medium (1 to 3 cm) in 30 patients, large (3 to 5 cm) in 43 patients, and massive (more than 5 cm) 32 patients. The tear involved the supraspinatus tendon in 44 patients; the supraspinatus and infraspinatus tendons in 76 patients.

Group 2 (control group) included 120 patients (45 men and 75 women; mean age: 63.91 years, range 38 to 78) (Table 1) who underwent arthroscopic meniscectomy for a meniscal tear with no history of rotator cuff symptoms.

These patients were frequency-matched by age (within 3 years) and gender with patients of Group 1.

Patients in group 1 were included in the study if they had a rotator cuff tear diagnosed on clinical and imaging grounds and a rotator cuff tear found at the time of surgery. Conservative management, including nonsteroidal anti-inflammatory drugs, physiotherapy and rest, failed in all patients, and they continued to experience unacceptable pain and weakness in the affected shoulder. None of the patients had undergone prior surgery on the affected shoulder. All patients fulfilled the following criteria: (1) positive rotator cuff lag signs on pre-operative examination (at least one among Jobe test, Napoleon test, lift-off test, and Patte test)[532] (2) no episodes of shoulder instability, (3) no radiographic sign of fracture of the glenoid or the tuberosities, (4) Magnetic resonance imaging (MRI) evidence of cuff tear, (5) rotator cuff tear of 1 or more tendons at arthroscopic examination, (6) no lesion of the glenoid labrum or of the capsule at arthroscopic examination.

Patients in group 2 were included in the study if they had a meniscal tear diagnosed on clinical and imaging grounds and a meniscal tear found at the time of surgery.

**Exclusion criterion for all participants were:** primary osteoarthritis of the operated or contralateral joint, previous operations on the shoulder or knee, inflammatory joint disease, hypertension, diabetes, or hypercholesterolemia managed with statins. Patients in Group 2 were also excluded from the study if they had have history of shoulder pain, or rotator cuff pathology diagnosed by imaging or on clinical grounds.

## Anthropometric measurements

We measured height and weight of every patient and calculated the Body Mass Index (BMI) the day of sampling (Table 1). The same examiner measured all the subjects before blood sampling was performed.

**Table 1:** Anthropometric measures (values in brackets are the range of values)

| | Group 1 (Patients with rotator cuff tears) | | Group 2 (Control group) | |
|---|---|---|---|---|
| **Gender** | **Male=45** | **Female=75** | **Male=45** | **Female=75** |
| **Height (m)** | 1.71 (1.54-1.87) | 1.58 (1.48-1.75) | 1.73 (1.5-1.9) | 1.60 (1.5-1.8) |
| **Weight (kg)** | 80.17 (60-110) | 69.61 (48-107) | 83.17 (61-109) | 69.18 (48-96) |
| **BMI** | 27.36 | 27.88 | 27.81 | 26.82 |
| **Age** | Male=63.42 (40-78) | Female=65.73 (48-83) | Male=63.31 (38-76) | Female=64.28 (50-78) |

## Measurement of total cholesterol and triglycerides

All blood samples were collected in an identical manner between 07.00 and 07.30 after an overnight fast started at 12.00 midnight. Biochemical analyses of blood were performed on fresh samples. Five millilitres of blood sample was taken from the patients into tubes (Vacutainer System, Becton Dickinson, NJ) and they were centrifuged at the relative cen-

trifugal force of *2750 for 10 min*. Sera were extracted from the samples and the concentrations of total cholesterol (TC) and triglycerides (TG) were measured by enzymatic methods with the CIBA Corning 550 Express Autoanalyzer (Boehringer Mannheim, Mannheim, Germany). Patients were considered to have established hypercholesterolaemia at levels > 6.2 mmol/L, and light hypercholesterolaemia at levels between 5.2 and 6.2 mmol/L [26]. Patients were considered to have established hypertriglyceridemia at levels > 4.5 mmol/L [26].

### Statistics

Data were entered in a commercially available database. Descriptive statistics were calculated, and analytical statistics were performed with non-paired sample *t*-test using Statistical Programs for the Social Sciences (SPSS). Significance was set at $P < 0.05$.

## RESULTS

The serum concentrations of triglyceride and total cholesterol were measurable in all patients. We were not able to determine any significant differences in serum concentrations of triglyceride and total cholesterol in patients with small, medium, large, and massive tears. Equally, there were no significant differences in serum concentrations of triglyceride or total cholesterol in patients with a supraspinatus tendon tear or supraspinatus and infraspinatus tendon tears. Therefore, for the purposes of this study, all tears were grouped together.

When comparing the two groups, no statistically significant differences either in triglyceride concentration ($P = 0.6$) or total cholesterol concentration ($P = 0.1$) were present (Tables 2 and 3).

**Table 2:** Levels of serum triglycerides (mg per decilitre) and millimoles per litre

| Serum triglycerides values | Group 1 (Patients with rotator cuff tears) | | | | Group 2 (Control group) | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | Male | | Female | |
| | mg per decilitre | millimoles per litre | mg per decilitre | millimoles per litre | mg per decilitre | millimoles per litre | mg per decilitre | millimoles per litre |
| **Mean** | 158.42 | 1.81 | 131.81 | 1.49 | 139.87 | 1.58 | 120.48 | 1.36 |
| **Median** | 129 | 1.48 | 125 | 1.41 | 130 | 1:47 | 103 | 1.16 |
| **SD** | 122.29 | 1.39 | 55.9 | 0.63 | 75.56 | 0.85 | 53.75 | 0.61 |
| **Range** | 47-853 | 0.53-9.64 | 41-312 | 0.46-3.52 | 54-464 | 0.61-5.24 | 41-260 | 0.46-2.94 |

**Table 3:** Levels of total serum cholesterol (mg per decilitre) and millimoles per litre

| Total serum cholesterol values | Group 1 (Patients with rotator cuff tears) | | | | Group 2 (Control group) | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | Male | | Female | |
| | mg per decilitre | millimoles per litre | mg per decilitre | millimoles per litre | mg per decilitre | millimoles per litre | mg per decilitre | millimoles per litre |
| **Mean** | 212.76 | 5.51 | 224.11 | 5.80 | 213.6 | 5.53 | 217.3 | 5.63 |
| **Median** | 212 | 5.49 | 228 | 5.91 | 217 | 5.62 | 213 | 5.52 |
| **SD** | 40.58 | 1.05 | 44.42 | 1.15 | 36.45 | 0.94 | 39.28 | 1.02 |
| **Range** | 140-308 | 3.62-7.98 | 126-344 | 3.26-8.91 | 134-286 | 3.47-7.40 | 142-314 | 2.68-8.13 |

### Group 1

In Group 1 (rotator cuff tears), triglyceride concentration was > 4.5 mmol/L in 1 patient. No patients were under treatment for high serum triglyceride levels. In the same group, total cholesterol concentration was > 6.2 mmol/L in 41 patients (34.1%). Light hypercholesterolaemia (5.2-6.2 mmol/L) was present in 42 patients (35%). No patients were under treatment for high serum cholesterol levels.

### Group 2

In Group 2 (control group), triglyceride concentration was > 4.5 mmol/L in 1 patient. No patients were under treatment for high serum triglyceride levels. In the same group, total cholesterol concentration was > 6.2 mmol/L in 33 patients (27.5%). Light hypercholesterolaemia (5.2-6.2 mmol/L) was present in 47 patients (39.2%). No patients were under treatment for high serum cholesterol levels.

## DISCUSSION

Patients with a rotator cuff tear showed no statistically significant difference in serum triglyceride and total cholesterol concentrations when compared to subjects of the same age and sex undergoing arthroscopic meniscectomy, and who had no history of rotator cuff injury. This is the first study, to our knowledge, to examine the serum triglyceride and total cholesterol concentrations in patients with rotator cuff tears. We already showed in a comparable, but not identical population, that normal, but in the high range of normal, increasing plasma glucose levels are associated to rotator cuff tears.

Strengths of the present study include the systematic collection of blood samples, the use of pre-operative imaging and of arthroscopy to diagnose rotator cuff and meniscal tears, and the relatively large sample size of our study group. Nevertheless, we acknowledge the cross-sectional nature of the present investigation, which cannot completely

resolve issues concerning temporality, or rule out other factors that may influence rotator cuff tendinopathy. Another limitation of our study was that we have no data about high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low density lipoprotein (VLDL) concentrations in our patients. More detailed analysis could reveal lipoprotein abnormalities. The association between LDL and HDL cholesterol and the development of coronary heart disease is well established [234], and the management of coronary heart disease has traditionally focused on reduction of LDL cholesterol or of the total lipid profile [563]. We do not know whether such strategies might exert a beneficial effect on tendon problems as well. We are fully aware that more anthropometric measures could be performed (for example, waist and hip girth, and skinfold measurements). Unfortunately, we did not collect these data in our patients: this could be the subject of future endeavours.

Ideally, the control group should have been constituted by healthy people. However, as this was a frequency-matched case-control study, if, on the one hand, it could have been relatively simple to find healthy young person, the same would have not applied for elderly person, especially given the stringent exclusion criteria in our study. Among the various diseases of the lower limb, we choose to enrol in the control group patients with pathology of the lower limb with a likely mechanic, not metabolic cause, different from tendon pathology. Classically, the causative mechanisms of rotator cuff pathology have been subdivided into extrinsic and intrinsic factors [463]. Intrinsic factors focus on the pathologic changes lying predominantly within the tendon itself. Extrinsic factors are variables which interact to contribute to rotator cuff damage. They can be broadly grouped into anatomical (acromial morphologic characteristics, *os acromiale* and acromial spurs) and environmental factors (shoulder overuse, smoking, and any medical condition that impairs the inflammatory and healing response such as diabetes mellitus) [28].

In a retrospective cohort study of 205 patients, Harvie et al [249] showed a higher risk of symptomatic full-thickness rotator cuff tears in siblings of patients with rotator cuff tears versus controls, implying a role for genetic factors [408]. There is an association between obesity and shoulder repair surgery in men and women aged 53 to 70, suggesting that increasing body-mass index is a risk factor for rotator cuff pathologies [670]. Obesity could contribute to decreased vascularity through its associations with risk factors for vascular disease, such as elevated cholesterol [305], atherosclerosis [270], diabetes, hypertension, metabolic syndrome and decreased physical activity [592]. A correlation between adiposity and rotator cuff tendinopathy has been proposed, but, while the association with body-mass index and tendinopathy has been reported [670], no studies focused on serum levels of lipids and rotator cuff tendinopathy.

A major histopathological feature of tendinopathy is a failed healing response. Accumulation of lipids and ground substance (glycosaminoglycans), and calcium deposits represent age-related changes of the tendon [297]. During ageing, lipid accumulation is extracellular: lipids with a high content of esterified cholesterol spread along the longitudinal axis of col-

lagen fibres. Lipid deposition disrupts the fibre bundles, and may through this mechanism decrease tendon strength [297].

The association between tendon injury and adiposity has been examined [210]. Elevated adiposity can be frequently associated with tendon injury [210], and it seems that elevated adiposity develops prior to tendon pathology, even though any definitive conclusion should be reached with caution [209]. Although some evidences suggest that there is a possible association between tendinopathy of the lower limb and high cholesterol levels [417, 485], we could not find similar results in our population of patients with rotator cuff tears. Moreover, while histopathological examination of specimens harvested during surgery for tendinopathy in the lower limb showed fatty degeneration or tendolipomatosis [296], we failed to show evidence of fatty degeneration in tendon samples from the rotator cuff and the long head of the biceps tendon. In addition, while there seems to be evidence a possible role of high serum lipid concentration and complete rupture of the Achilles tendon [417, 485], our data suggest no role of the serum cholesterol and triglyceride concentration in rotator cuff tears. We can speculate that, probably, the mechanisms underlying tendinopathy may be different in the lower or upper limbs, even though more studies are needed to confirm this preliminary statement.

Some authors [342] proposed the use of dietary supplements, including Omega 3 fatty acids and antioxidants, in the management of tendinopathies, on the basis that high levels of cytokines, (i.e. pro-inflammatory interleukin 1b and vascular endothelial growth factor), have been reported in the bursa of patients with rotator cuff pathology. The potential benefits of dietary supplementation in the management of tendinopathy [342, 424] need further research using appropriately designed adequately powered randomised controlled trial studies, with both objective and patient-centred outcome measures.

On the basis of our study, we doubt that triglycerides serum levels and total serum cholesterol have a causative role in the pathogenesis of rotator cuff tears, even though we advocate more research to reach definitive conclusion. The study of the different fractions of serum cholesterol may shed further light, and is the subject of future research.

In conclusion, there appears to not be an association between serum lipids level and rotator cuff tears. Additional research is required to understand the causative role, if any, of serum triglyceride and total cholesterol concentrations in rotator cuff pathology.

# Randomised controlled trials to improve rotator cuff healing

# Chapter 9

## Equivalent clinical results of arthroscopic single row and double row suture anchor repair for rotator cuff tears a randomized controlled trial

## ABSTRACT

**Background:** Restoring of anatomic footprint may improve the healing and mechanical strength of repaired tendons. A double row of suture anchors increases the tendon-bone contact area, reconstituting a more anatomic configuration of the rotator cuff footprint.
**Hypothesis:** No difference in clinical and imaging outcome between single row and double row suture anchor technique repairs of rotator cuff tears.
**Study Design:** Randomized controlled clinical trial. Level of evidence 1.
**Methods:** We recruited 60 patients. In 30 patients, rotator cuff repair was performed with single row suture anchor technique (Group 1). In the other 30 patients, rotator cuff repair was performed with double row suture anchor technique (Group 2). 8 patients (4 in the single row anchor repair group and 4 in the double row anchor repair group) were lost at follow up.
**Results:** 8 patients did not return at the final follow up. At the 2 year follow-up, no statistically significant differences were seen with respect to the UCLA score and ROM values. Post-operative MR arthrography at 2 years of follow up in group 1 showed intact tendons in 14 patients, partial thickness defects in 10 patients and full thickness defects in 2 patients. In group 2, MR arthrography showed an intact rotator cuff in 18 patients, partial thickness defects in 7 patients, and full thickness defects in 1 patient.
**Conclusions:** Single and double row technique provide comparable clinical outcome at 2 years. A double row technique produces a mechanically superior construct compared to the single row method in restoring the anatomic footprint of the rotator cuff, but these mechanical advantages do not translate in superior clinical performance.

## INTRODUCTION

Arthroscopic management of rotator cuff tears has evolved from simple debridement to arthroscopic repair providing anatomic reconstruction [92,93,587].

Restoring the anatomic footprint may improve the healing and mechanical strength of repaired tendons [32]. A single row of suture anchors may not be effective for this purpose. A double row of suture anchors increases the tendon-bone contact area, reconstituting a more anatomic configuration of the rotator cuff footprint.

We evaluated the results of a randomized controlled trial of arthroscopic repair in patients with large and massive rotator cuff tears in whom the repair was effected using single or double row arthroscopic technique.

## MATERIALS AND METHODS

Our institutional review board approved the study, and all patients gave written informed consent to participate in this clinical trial.

### Eligibility criteria

Patients were included in the study if they had a rotator cuff tear diagnosed on clinical grounds, no episodes of shoulder instability, no radiographic signs of fracture of the glenoid or the greater or lesser tuberosity, magnetic resonance imaging evidence of cuff tear, duration of symptoms of at least three months, inadequate response to non-operative management (including non-steroidal anti-inflammatory drugs, physiotherapy, rest and one local cortico-steroid injection), an unretracted and sufficiently mobile full-thickness rotator cuff lesion to allow a double row repair found at the time of surgery.

Patients were excluded from the study if they had inflammatory joint disease, retracted and insufficiently mobile lesions to allow a double row repair found at the time of surgery, prior surgery on the affected shoulder and inability to complete questionnaires because of language problem or cognitive disorder.

### Recruitment and randomisation

Patients were recruited among those referred by primary care doctors because of symptoms of rotator cuff tears. Eligible patients were enrolled by the examining orthopaedic surgeon. Each patient was given full verbal and written information about the trial, and written informed consent was obtained by the operating surgeon.

Recruitment started in February 2004 and was completed in September 2004. Of 197 patients screened for eligibility, 60 patients were eligible and were randomized; 30 patients to single row anchor repair and 30 patients to double row anchor repair. All patients received the allocated treatment.

Of 60 participants randomized to one of the two treatments, two year results were available for 52. 8 patients (4 in the single row anchor repair group and 4 in the double row anchor repair group) did not return at the final follow up (Fig 1).

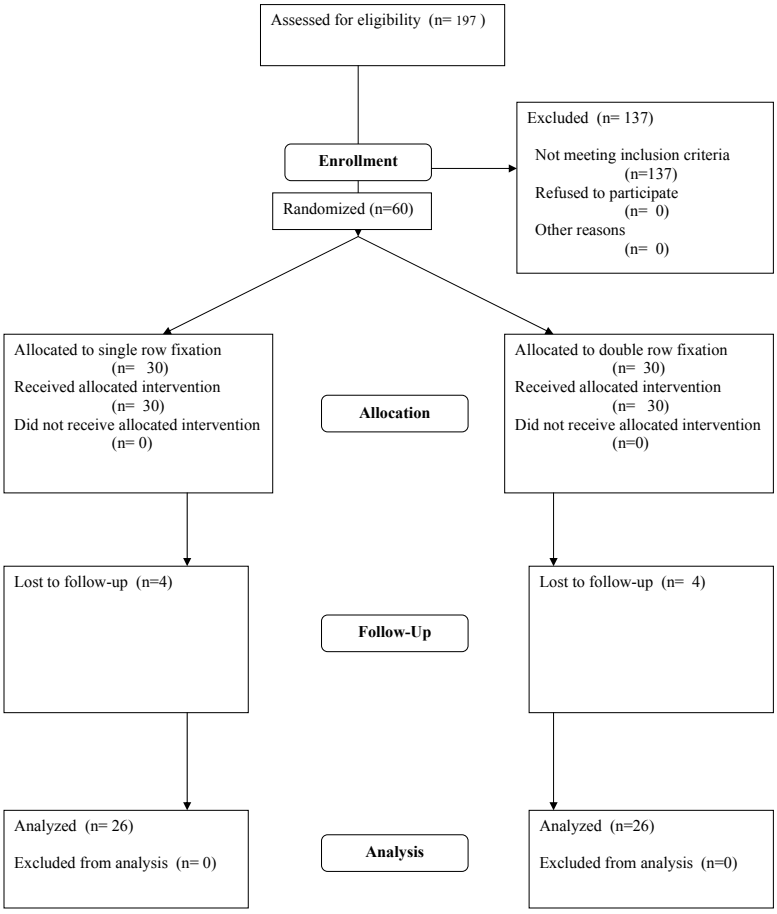


**Figure 1:** Patients' consort statement.

## Evaluation

We performed pre-operative evaluations the day before surgery, and report the results of post-operative evaluation at a final follow at an average of 22.5 months (range 18 to 25 months) from the operation. Each patient was evaluated for arm dominance, trauma his-

tory, duration and type of preoperative symptoms, type of lesion, pre- and post-operative range of motion (ROM), pre- and post-operative modified shoulder score (UCLA).

## Imaging

All patients received a standard pre-operative assessment using standard radiographs (antero-posterior projections, neutral, external and internal rotation, a lateral view of the scapula, and an axillary view) and MRI scans. Oblique coronal, oblique sagittal and axial T2-weighted spin-echo MRIs (repetition time: 3,200 milliseconds; echo time: 85 milliseconds) were obtained in all patients.

All patients received a post-operative MR arthrography at the final follow up appointment. The joint was injected with 1.5 mL of gadolinium and 8.5 mL of normal saline solution under fluoroscopic control. MRI imaging was performed with a 1.5-T scanner. We employed T2-weighted, gradient-echo (GE) Spectral Presaturation Inversion Recovery (SPIR) sequences in true axial scans and T1-weighted, GE SPIR sequences in oblique coronal planes that are parallel to the course of the supraspinatus muscle, and oblique sagittal planes that are parallel to the glenoid fossa. Imaging time was approximately 18 minutes. All MR arthography was performed and evaluated by the same fully trained board certified radiologist with a special interest in muscolo-skeletal imaging.

The rotator cuff was reported as intact (Fig 2) or torn using previously published MR arthrography criteria. Partial tears (Fig 3) were diagnosed in the presence of contrast fluid signal filling a partial tendon defect. Full thickness tears (Fig 4) were diagnosed in the presence of extravasation of contrast material to the subacromial- subdeltoid space [699].

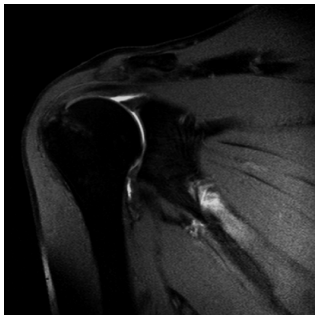

**Figure 2:** Coronal T1-weighted image showing intact tendon after single row repair.

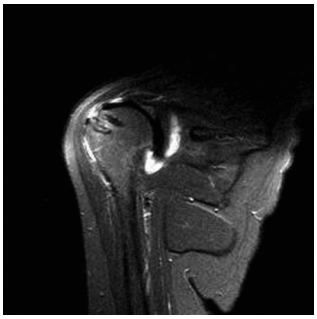

**Figure 3:** Coronal T1-weighted image showing partial rotator cuff tear with delamination. Note the absence of contrast material in the subacromial-subdeltoid space after double row repair.

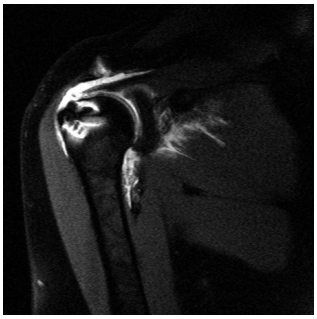

**Figure 4:** Coronal T1-weighted image showing a moderate fluid accumulation in the subacromial-subdeltoid bursa, a finding indicative of a full-thickness tear at the attachment site after double row repair.

### Functional assessment

A modified UCLA (University of California, Los Angeles) shoulder rating scale was used to evaluate preoperative and postoperative shoulder pain, function and range of motion, strength and patient satisfaction. The maximum score obtainable is 35, and the results were classified as excellent (34-35 points), good (28-33), fair (21-27), or poor (0-20).

### Range of motion

A standard universal goniometer was used for measurement with scales marked in one-grade increments. Patients were positioned supine on an examining couch with the shoulder at 90° of abduction in the scapular plane (approximately 15° anterior to the coronal plane). Measurement of supine forward elevation (sagittal plane), internal and external rotation (90° abduction) were obtained using standard measurement guidelines. Care was taken to fix the scapula with one hand while the other hand of the examiner's rotated the shoulder into position. One examiner (LR) held the shoulder position, while a second examiner (UGL) obtained the measurement after a firm endpoint was established. The forearm was held in neutral rotation during rotational measurement. Three measurements were taken for each shoulder, and the mathematical average used for statistical purposes.

### Randomization procedure

After a diagnostic arthroscopy assessing the status of the shoulder joint, and the presence and the size of the rotator cuff tear, we ascertained whether the tear was mobile evaluating the medial-to-lateral and anterior-to- posterior mobility of the tear margins using a soft tissue grasper. If this was the case, at that stage patients were randomized into one of two groups, to receive either single row suture anchor repair technique (Group 1), or double row suture anchor repair technique (Group 2).

We used a random-numbers table to allocate subjects. Starting with an arbitrary point in the table, we selected 52 sequential random numbers. The first 26 numbers were assigned to the single row group, and the next 26 were assigned to the double row group. These assignments were then arranged in an ascending order. This procedure produced a random sequence of consecutive treatment allocations. Sealed, opaque numbered envelopes containing the treatment assignments were prepared, with care being taken to make sure that the order of the envelopes exactly matched the allocation schedule.

All surgical interventions were performed by the same surgeon (FF). After diagnostic arthroscopy, the extent of the tear was assessed, the tendon margins were debrided, and a bone bed was prepared using a power shaver so as not to decorticate the bone. The

rotator cuff tears were classified according to their size, shape and location. At that time, the envelope was opened, and the patient allocated to either Group 1 (single row), or Group 2 (double row).

In Group 1 (single row suture anchor repair technique), there were 12 men and 14 women (mean age: 63.5 years; range 43 to 76). The dominant arm was affected in 20 patients. The rotator cuff tears were classified as large (3 to 5 cm) in 18 patients, and massive (> 5 cm) in 8 patients. There were 12 crescentic lesions, 4 L-shaped lesions, and 10 U-shaped lesions. The tear involved the supraspinatus tendon in 12 patients; the supraspinatus and infraspinatus tendons in 11 patients and the supraspinatus and subscapularis tendons in 3 patients.

In Group 2 (double row suture anchor repair technique), there were 16 men and 10 women (59.6 years, range 45 to 80). The dominant arm was affected in 19 patients. The rotator cuff tears were classified as large (3 to 5 cm) in 21 patients, and massive (> 5 cm) in 5 patients. There were 14 crescentic lesions, 6 L-shaped lesions, and 6 U-shaped lesions. The tear involved the supraspinatus tendon in 15 patients; the supraspinatus and infraspinatus tendons in 9 patients; and the supraspinatus and subscapularis tendons in 2 patients.

**Arthroscopic technique**

Patients underwent brachial plexus block associated in seven cases with general anaesthesia, and were placed in a lateral decubitus position. The arm was suspended at approximately 45° of abduction and 20° of forward flexion. Distraction of the shoulder joint was accomplished with 4.5 to 6.5 kg of traction. Four to six portals were used. A posterior portal was produced, and the arthroscope was inserted into the glenohumeral joint. A diagnostic arthroscopy was then performed to evaluate the extent of the rotator cuff tear, any lesions of the biceps tendon, and other associated lesions. The main subacromial portals were the postero-lateral viewing, the antero-lateral, and the lateral working portal, with an 8.25 mm cannula. To control bleeding, we used radiofrequency, adrenalin admixture to the irrigation fluid, and asked the anesthesiologist to lower the systolic blood pressure to 90 mm Hg if possible. An arthroscopic pump maintained fluid pressure at 40 mmHg, increasing it temporarily on demand.

A spinal needle was introduced percutaneously to determine the precise location for placement of the anterolateral portal produced approximately 2 to 3 cm anterior and lateral to the anterolateral corner of the acromion. If the subscapularis tendon was involved, an anterior midlateral portal was produced just superior to the lateral half of the subscapularis tendon. The lateral portal was used to mobilize the rotator cuff back to its bony insertion. The mobility of the rotator cuff was assessed.

Using a burr through the lateral portal, the footprint of the greater tuberosity was abraded.

The single row anchor repair was performed placing one row of suture anchors double loaded with N° 2 Fiberwire (Biocorkscrew, Arthrex) just in the lateral aspect of the footprint.

The double row anchor repair technique was performed as described previously. Briefly, one row of anchors was placed in the medial aspect of the footprint, just lateral to the articular surface of the humeral head. A lateral row of anchors was then placed on the lateral aspect of the footprint, slightly proximal to the greater tuberosity (Fig 5-6). The anchors used were Biocorkscrew (Arthrex) double loaded with N° 2 Fiberwire (Arthrex).

The number of suture anchors varied with the size of the tear and the type of repair techniques: we used 1.9 (range 1 to 2) anchors in the Group 1 (single row), and 2.3 anchors (range 2 to 4) in the Group 2 (double row).

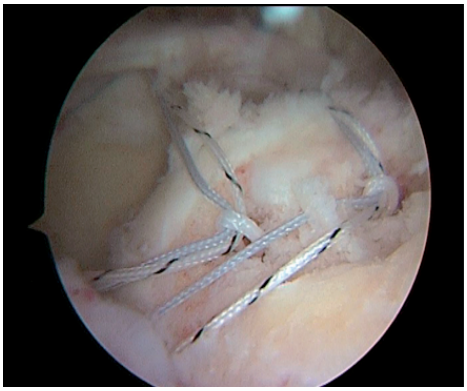

**Figure 5:** Arthroscopic view from a posterolateral portal showing the insertion of medial and lateral double loaded anchors adjacent the articular margin of the humeral head.

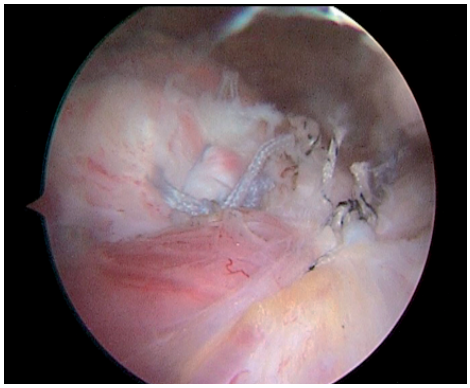

**Figure 6:** Arthroscopic view from a posterolateral portal showing a completed double row repair after knot tying.

L-shaped and U-shaped tears were first repaired with a side to side suture providing margin convergence of the two edges of the cuff, before fixation of the cuff to bone.

## Postoperative management

Postoperative management was the same for both groups. The arm was supported using a sling with an abduction pillow for 6 weeks. Active elbow flexion and extension were allowed, but terminal extension was restricted. Passive external rotation was started from the first day after surgery, and maintained within a comfortable range. Overhead stretching was restricted until 6 weeks postoperatively to avoid damaging the repair. At six weeks, the sling was removed, and overhead stretching with a rope and pulley were started. Isoinertial strengthening and rehabilitation of the rotator cuff, deltoid and scapular stabilizers were initiated at 10 or 12 weeks after the operation. Rehabilitation was continued for 6

months. Heavy manual work and overhead activities were allowed after a good restoration of shoulder strength, which occurred 6 to 10 months after surgery.

### Statistics

Statistical analyses were blinded and performed according to the 'intention-to-treat' principle. Descriptive statistics were calculated. The results of surgery in the two groups were compared using the Wilcoxon Sign Rank test. Significance was set at $P < 0.05$.

## RESULTS

No infection, neurological or vascular complications were experienced.

**Group 1** (single row suture anchor repair technique) (Table 1). The UCLA rating system showed a statistically significant improvement from a preoperative average rating of 11.5 (range 6 to 14) to an average of 32.9 (29-35) postoperatively ($P < 0.05$). Forward flexion averaged 110° (range, 30°-140°) preoperatively and 159° (range 150° to 170°) at final follow up ($P < 0.05$). The average external rotation improved from 83.2° (range 65° to 95°) preoperatively to 132.4° (range 90° to 140°) at final follow up ($P < 0.05$).

Internal rotation increased from a mean of 27.3° (range 20° to 33°) preoperatively to a mean of 37.3° (range 27° to 42°) at final follow up ($P < 0.05$).

Post-operative MR arthrography examination at 2 years of follow up showed intact tendons in 14 patients, partial-thickness defects in 10 patients, and full-thickness defects in 2 patients (Table 2).

**Group 2** (double row suture anchor repair technique) (Table 1). The UCLA rating system showed a statistically significant improvement from a preoperative average rating of 10.1

**Table 1:** Clinical findings.

| **Group 1 (single row)** | Preoperative | Postoperative |
|---|---|---|
| UCLA | 11.5 (6-14) | 32.9 (29-35) |
| Forward flexion | 110° (range, 30°-140°) | 159° (range, 150°-170°) |
| External rotation | 83.2° (range 65°-95°) | 132.4° (range 90°-140°) |
| Internal rotation | 27.3° (range 20°-33°) | 37.3°(range 27°-42°) |

| **Group 2 (double row)** | Preoperative | Postoperative |
|---|---|---|
| UCLA | 10.1 (5-14) | 33.3 (30-35) |
| Forward flexion | 100° (range, 30°-150°) | 156° (range, 140°-170°) |
| External rotation | 79.6° (range 62°-93°) | 131.3° (range 85°-137°) |
| Internal rotation | 28.6° (range 22°-35°) | 40.3°(range 26°-43°) |

**Table 2:** Findings at MR arthrography.

| | GROUP 1 (single row) | GROUP 2 (double row) |
|---|---|---|
| **Intact** | 14 | 18 |
| **Partial thickness defect** | 10 | 7 |
| **Full thickness defect** | 2 | 1 |

(range 5 to 14) to an average of 33.3 (range 30 to 35) postoperatively ($P < 0.05$). Forward flexion averaged 100° (range 30° to 150°) preoperatively and 156° (range 140° to 170°) at final follow up ($P < 0.05$).

The average external rotation improved from a mean value of 79.6° (range 62° to 93°) preoperatively to 131.3° (range 85° to 137°) postoperatively ($P < 0.05$).

Internal rotation increased from a mean of 28.6° (range 22° to 35°) preoperatively to a mean of 40.3°(range 26° to 43°) at final follow up ($P < 0.05$).

Postoperative MR arthrography at final follow up showed an intact rotator cuff in 18 patients, partial-thickness defects in 7 patients, and full-thickness defects in 1 patient (Table 2).

There was no statistically significant difference in total postoperative UCLA scores when comparing single row suture anchor repairs versus double row suture anchor repairs.

The ROM did not differ between the two groups: it improved in all the directions measured ($P > 0.05$).

## DISCUSSION

We compared the clinical and imaging outcome of large and massive rotator cuff tears repaired arthroscopic using a standard single or double row technique. In our hands, single and double row technique provide comparable clinical outcome. Compared to the single row method, a double row technique produces a mechanically superior construct in restoring the anatomic footprint of the rotator cuff, but these mechanical advantages do not translate in superior clinical performance.

To our knowledge, this is the first randomized controlled trial to compare the outcome of arthroscopic single row or double row anchor suture repair rotator cuff surgery using both clinical and imaging criteria. We acknowledge that we did not perform a formal power analysis, and that we planned the choice of the number of patients to enrol in the study according to what we knew our unit could deliver within the time which we chose to allocate to the study. However, despite this partial weakness of the present investigation, our selection and recruitment process, our assessment criteria and our follow up were extremely rigorous, and performed in strict scientific fashion. Also, with the numbers of patients enrolled, the results of our study are univocal.

We used MR arthrography to evaluate the anatomical appearance following operative rotator cuff repair because this technique affords several advantages over conventional MR imaging, including better definition of the rotator cuff and tendon defects, and a better differentiation of rotator cuff degeneration from partial or complete rotator cuff tears [613].Other strengths of this study include the use of a single surgeon, and its prospective randomized nature.

We followed up patients clinically and with imaging for 22.5 months. Although this may be considered a relatively short time, we believe that, by then, the results of surgery would have stabilized, and recovery effected. Also, this length of follow up was chosen because we wished to minimize the number of patients defaulting from the study: we felt that it would have been difficult to ask patients to return for assessment several years later for clinical assessment and imaging.

It is difficult to compare the findings of the present study with those of previous reports, as we know of no other prospective studies performed using of MRI arthrography to compare the clinical and anatomical outcome of large and massive rotator cuff tears repaired arthroscopic using single or double row anchors.

Biomechanical studies comparing single versus double row suture anchor technique for rotator cuff repair show that a double row of suture anchors increases the tendon-bone contact area and restores the anatomic rotator cuff footprint, providing a better environment for tendon healing [32].

Both the transosseous technique and the arthroscopic single-row fixation technique restore respectively 85% and 65% of the normal surface area, failing to restore the normal footprint of the supraspinatus tendon to the greater tuberosity [32]. Double row suture anchor fixation fully reproduces the original supraspinatus footprint [304], decreases the gap formation and strain over the footprint, and improves its initial strength and stiffness when compared to a conventional single-row repair [617]. Single row repairs were similar to double row repairs in load to failure, cyclic displacement and gap formation [304].

In comparative retrospective studies, the clinical outcome of single row and double row anchor suture technique were comparable, though rotator cuff integrity was more likely to be maintained with double row repair [678]. Sugaya *et al* [617] performed the above study using MRI, which is sensitive and specific for diagnosis of full-thickness tears, but has a sensitivity of only 20% for partial-thickness tears [613]. Instead, MRI arthrography is more sensitive for this purpose [613].

Rotator cuff surgery aims to provide tendon fixation secure enough to hold the repaired tendon in place until biological healing occurs. Several factors may be implicated in failure of rotator cuff repairs, including suture or knot failure, inadequate tendon to bone fixation, and lack of tendon to bone healing. As we re-insert tendinous tissue into bone, theoretically only the re-constitution of enthesial fibrocartilage would guarantee an optimal outcome [643].

The concept of restoration of the anatomical footprint is appealing, but we did not find any statistical difference between the two techniques. Also, double row repair requires longer surgical time, is more expensive as a greater number of suture anchors is required, and may well be technically more demanding.

In conclusion, our study shows that there are no advantages in using a double row suture anchor technique to restore the anatomical footprint. The mechanical advantages evidenced in cadaveric studies do not translate into superior clinical performance when compared with the more traditionally, technically less demanding, and economically more advantageous technique of single row suture anchor repair.

# Chapter 10

## Platelet-rich plasma augmentation for arthroscopic rotator cuff repair: a randomised controlled trial

## ABSTRACT

**Background:** Following reinsertion on the humerus the rotator cuff (RC) has limited ability to heal. Growth factors augmentation has been proposed to be able to enhance healing in such procedure.

**Purpose:** To assess the efficacy and safety of the addition of growth factor augmentation during rotator cuff repair.

**Study Design:** Randomised controlled trial; Level of evidence, 1.

**Methods:** Eighty-eight patients with a rotator cuff tear were randomly assigned by a computer-generated sequence to receive arthroscopic rotator cuff repair without (n = 45) or with (n = 43) augmentation with autologous platelet-rich fibrin matrix (PRFM). The primary endpoint was the post-operative difference in the Constant score between the 2 groups. The secondary endpoint was the integrity of the repaired rotator cuff, as evaluated by MRI. Analysis was on an intention to treat basis.

**Results:** All the patients completed follow-up at 16 months. There was no statistically significant difference in total Constant Score when comparing the results of arthroscopic repair of the 2 groups (95% confidence interval [CI], −3.43 - 3.9) ($P = 0.44$). There was no statistically significant difference in MRI tendon score when comparing arthroscopic repair with or without PFRM ($P = 0.07$).

**Conclusions:** Our study does not support the use of autologous PRFM for augmentation of a double row repair of a small or medium RC tear to improve the healing of the RC. Our results are applicable to small and medium RC tears: it is possible that PRFM may be beneficial for large and massive RC tears. Also, given the heterogeneity of PRFM preparation products available on the market, it is possible that other preparations may be more effective.

## INTRODUCTION

Rotator cuff (RC) tendon tears account for more than 4.5 million physician visits per year, and over 250,000 RC repair surgeries performed annually in the United States [684]. The pathogenesis of RC tears is debated. The RC has limited ability to heal back to its insertion on the humerus following repair, possibly because of the poor vascularization of tendon tissue, and also because the histopathological changes which accompany a rupture are localized not only at the site of rupture but also in the macroscopic intact tendon portion, suggesting more generalised involvement of the tendon. Given this limited ability for healing, several strategies - including growth factors and cytokines, gene therapy, tendon augmentation graft and tissue engineering with mesenchymal stem cells - have been proposed to enhance tendon healing. Several growth factors are upregulated during RC healing, and they may be used to augment RC repairs.

Platelet-rich plasma (PRP) and platelet-rich fibrin matrix (PRFM), or autologous platelet-derived growth factors, are bioactive components of whole blood, which are now being widely tested in different fields of medicine to aid healing in tissue with poor healing potential [189, 193, 244, 322, 442, 443, 552-554]. Cascade® Autologous Platelet System (MTF, Musculoskeletal Transplant Foundation) is a completely autologous platelet biologic matrix [414], with a high concentration of viable platelets, extracted from a small amount of the patient's own blood, spun through a centrifugation process and resulting in a dense suturable PRFM that can be delivered directly to the tear site and sutured in place to potentially stimulate a reparative healing response for soft tissue and bone repair.

To date, there are no data from randomised trials assessing the efficacy and safety of PRFM for augmentation of RC repair. We therefore performed a randomized controlled trial to compare the efficacy and safety of augmentation with PRFM for arthroscopic RC repair compared with non-augmented repair of the RC, to test the hypothesis that augmentation with PRFM would result in increased improvement in shoulder function and better MRI imaging in patients undergoing surgical repair of small and moderate RC tears.

## METHODS

Our institutional review board approved the study (ISRCTN.org - number, ISRCTN49643328). Recruitment started in January 2007 and was completed in April 2008. Eligibility criteria are reported in Table 1.

88 patients were eligible and were randomized: 45 patients to arthroscopic RC repair without augmentation with PFRM (group 1), and 43 patients to arthroscopic RC repair and augmentation with PFRM (group 2). All patients received the allocated treatment. Of

**Table 1:** Eligibility Criteria

| | |
|---|---|
| **Inclusion Criteria** | Rotator Cuff tear diagnosed on clinical grounds |
| | Isolated supraspinatus tear |
| | Failure of 6 months of conservative treatment |
| | No episodes of shoulder instability |
| | No radiographic signs of fracture of the glenoid or the greater or lesser tuberosity |
| | Magnetic resonance imaging evidence of cuff tear |
| | A repairable full-thickness tear of the RC found at the time of surgery |
| | Associated pathology of the long head of the biceps |
| **Exclusion Criteria** | Inflammatory joint disease |
| | Irreparable full-thickness tear or partial thickness tear of the RC found at the time of surgery |
| | Symptomatic arthritis of the acromioclavicular joint |
| | Rotator cuff arthropathy |
| | Pathologies of the subscapularis tendon |
| | Workers' Compensation claims |
| | Prior surgery on the affected shoulder |

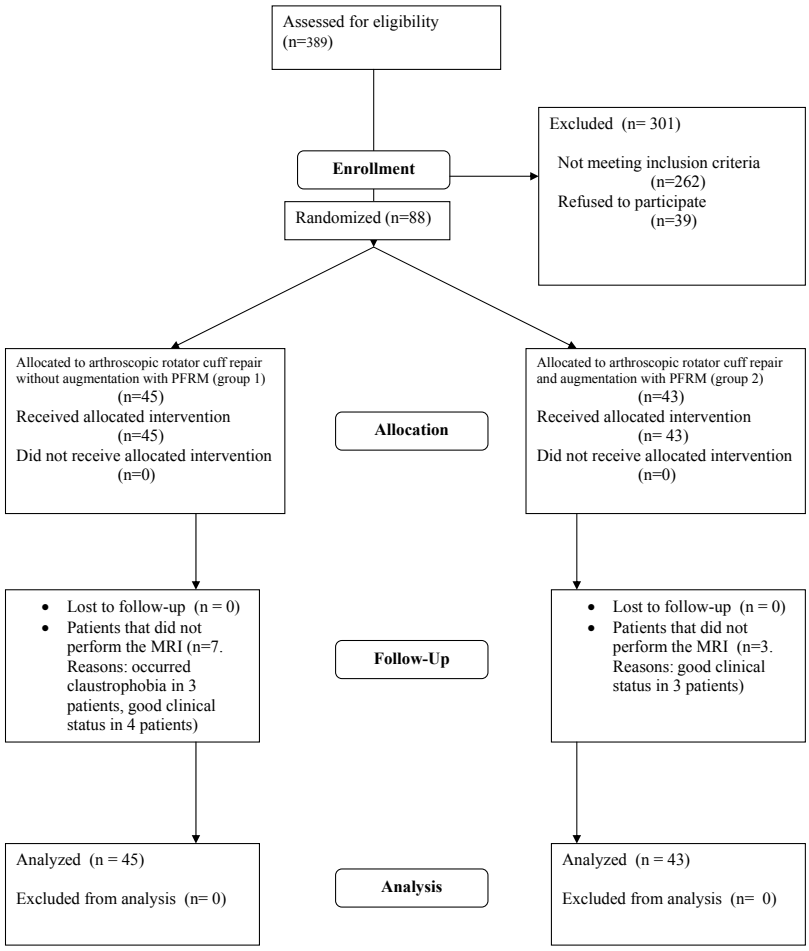


**Figure 1:** CONSORT (Consolidated Standards of Reporting Trials) flowchart.

participants randomized, at least 16 month clinical results were available for all patients, and radiological results for 78 (Figure 1).

## Endpoints

The primary endpoint was the difference in change from baseline to 16 months in the Constant score between the 2 groups. The secondary endpoint was the integrity of the repaired RC, as evaluated by MRI. All adverse events and serious adverse events were reported; investigators assessed whether they were related to procedure. Investigators informed the local ethical committees/institutional review board of any serious adverse events or serious adverse effects.

## Evaluation

We performed pre-operative evaluations the day before surgery, and reported the results of post-operative evaluation at a final follow at an average of 20.2 months (range 16 to 30 months) from the operation. Each patient was evaluated for pre- and post-operative Constant-Murley scoring system [130]. Nonarthrographic MRI studies were performed on all patients pre - and post-operatively at the final follow up appointment [691]. Oblique coronal, oblique sagittal and axial T2-weighted spin-echo MRIs (repetition time: 3,200 milliseconds; echo time: 85 milliseconds) were obtained in all patients. We employed T2-weighted, gradient-echo (GE) Spectral Presaturation Inversion Recovery (SPIR) sequences in true axial scans and T1-weighted, GE SPIR sequences in oblique coronal planes that are parallel to the course of the supraspinatus muscle, and oblique sagittal planes that are parallel to the glenoid fossa. Imaging time was approximately 18 minutes per patient.

All scans were evaluated independently by 2 orthopaedic surgeons who received specific training in shoulder MRI and were blinded to patients' clinical information and surgical history. Disagreements were discussed in a consensus meeting, where the scans were re-evaluated and a final decision was made [97]. Post-operative scans were evaluated for the presence of a full-thickness tear, defined as absence of visible tendon fibres extending across the entire tendon from inferior to superior [97] (for details, please refer to Table 4). Tendon signal intensity was divided into 3 grades [606] (for details, please refer to Table 4). On this scale, a tendon repair with a completely normalized appearance had a score of 9, while 3 is the worst possible score.

The Constant-Murley scoring system shoulder rating scale was used to evaluate preoperative and postoperative shoulder pain (15 points), activities of daily living (20 points), range of movement (40 points), and power (25 points). The total possible score is 100 points, indicating an asymptomatic and healthy person, while the worst score is 0 points [130].

For muscle strength evaluation, we used a digital dynamometer (Myometer 500 N Athlantech Medical Devices-Notthingam). The mean value of 3 repeated measurement at 90° of elevation in the scapular plane was recorded and used for scoring strength in the Constant-Murley score [661]. A standard universal goniometer was used for measurement with scales marked in one degree increments. Three measurements were taken for each shoulder, and the mathematical average used for statistical purposes.

Rotator cuffs without a recurrent tear were evaluated for tendon's thickness, coverage of the greater tuberosity, and the intensity of the signal [2]. Each of these parameters was also graded numerically on a scale from I to III. Tendon thickness was compared with normal tendon using a division in 3 grades: grade I normal thickness, grade II more than 50%, and grade III less than 50%. The size of the supraspinatus tendon footprint was compared with the size of the footprint of a normal supraspinatus tendon, which covers the entire greater tuberosity from medial to lateral. In cases where the tendon attachment was medialized, the width of the medialized footprint was compared with width of the greater tuberosity. Grade III coverage was 3/3, grade II coverage was 2/3, and grade I coverage was 1/3 of the greater tuberosity.

Tendon signal intensity was divided into 3 grades [32]. Grade I: if the tendon evidenced a light and diffused increase of the signal (different from that of the synovial fluid). Grade II: if the tendon appeared undamaged but there was a focal increase of the signal (the same as that of the synovial fluid) on the bursal or articular side. Grade III: if the increase of the signal's intensity (the same as that of the signal of the synovial fluid) involved the entire thickness of the tendon, with or without tendinous retraction. A tendon repair with a completely normalized appearance had a score of 9, with 3 being the worst possible score.

**Randomization procedure**

After a diagnostic arthroscopy assessing the status of the shoulder joint and the presence and the size of the RC tear, we ascertained whether the tear was mobile evaluating the medial-to-lateral and anterior-to-posterior mobility of the tear margins using a soft tissue grasper. If this was the case, at that stage patients were randomized into one of two groups. We used a random-numbers table to allocate subjects. Starting with an arbitrary point in the table, we selected 88 sequential random numbers. The first 45 numbers were assigned to the group one, and the next 43 were assigned to the group two. These assignments were then arranged in an ascending order. This procedure produced a random sequence of consecutive treatment allocations. Sealed, opaque numbered envelopes containing the treatment assignments were prepared, with care being taken to make sure that the order of the envelopes exactly matched the allocation schedule.

### Production of platelet rich fibrin matrix

Nine mL of venous blood are drawn with an aseptic technique from the antecubital vein by standard venipuncture using a sterile vacuum tube containing trisodium citrate and a thixotrophic polyester separator gel. The red blood cells and platelet-rich plasma (PRP) are separated by spinning the tube for 6 minutes in a standard centrifuge at 1,100 rounds per minute. The supernatant PRP is transferred from the first tube into a 35 mm Wheaton bottle, containing calcium chloride (1.0 M), using a 20 mL syringe and a 19G needle. The Wheaton bottle is placed back into the centrifuge equipped with a flat carrier-container and spun at a higher g force (4,500 RCF) for 25 minutes. A flat, circular membrane of platelet rich fibrin matrix (PRFM) is formed at the bottom of the container as it is spun using radial centrifugation. The final product is a membrane of autologous suturable fibrin which must be used within 30 minutes [414].

### Surgical technique

All arthroscopies were performed by the same fully trained surgeon. The greater tuberosity was decorticated with a motorized shaver. The RC was repaired with a double-row technique. The medial row consisted of 1 metal suture anchor (Fastin ® RC Anchor w/#2 ETHIBOND Excel, 5 mm, DePuy Mitek, Raynham, Massachusetts) (Figure 2) placed at the articular margin of the humeral head in a mattress fashion. Subsequently, a lateral row of anchors was inserted in the lateral aspect of the greater tuberosity. One of the suture limbs was used to position the PRFM under the supraspinatus tendon, above the bleeding surface of the greater tuberosity. The limb of the suture coming out through the cannula in the lateral portal was passed through the PRFM using a free needle (Figure 3), and reinserted via the cannula after

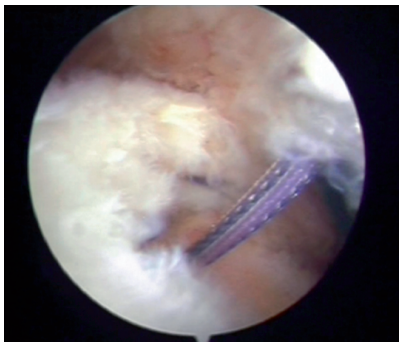

**Figure 2:** Arthroscopic view of a rotator cuff tear. A metal suture anchor (Fastin® RC Anchor w/#2 ETHIBOND Excel, 5 mm, DePuy Mitek, Raynham, Massachusetts) was placed at the articular margin of the humeral head

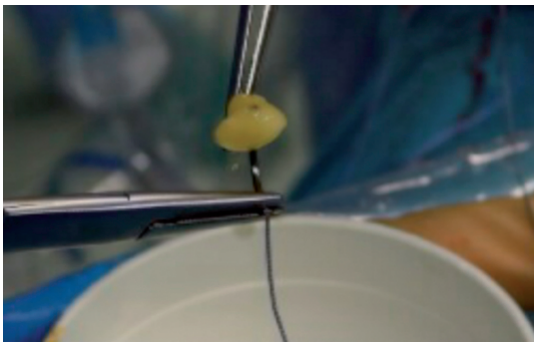

**Figure 3:** The limb of the suture coming out through the cannula in the lateral portal was passed through the PRFM using a free needle

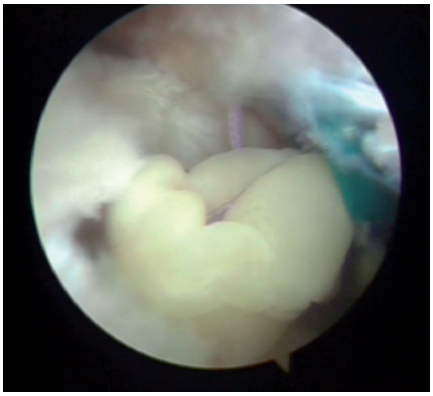

**Figure 4:** The PRFM was introduced into the shoulder joint

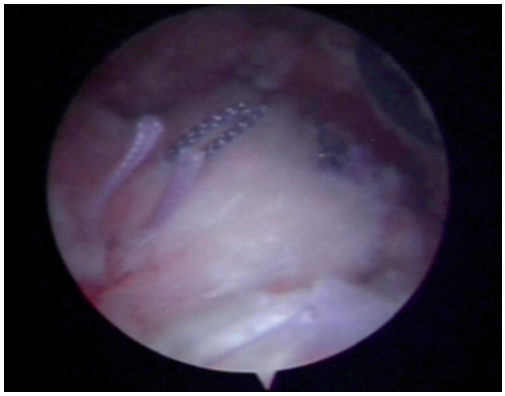

**Figure 5:** Subacromial view of the final stage of the repair

removing the rubber diaphram. One or two passages were generally sufficient. The PRFM was brought inside the joint by traction on the other end of the suture (Figure 4). With a suture passer, the portion of the suture which contained the implant was passed through the supraspinatus. The lateral sutures were tied using a sliding knot with 3 alternating half-hitches. The medial sutures, which passed through the implant, were tied with a non-sliding knot to prevent damage to the implant itself, in a mattress configuration (Figure 5).

All tenodeses were performed using an established tecnique. One of the suture limbs from one of the medial anchors was passed through the biceps tendon and then through the rotator cuff. The remaining intra-articular tendon stump of the biceps was resected in all the patients.

The operated shoulder was immobilized for 3 weeks using a sling with an abduction pillow. Pendulum exercises were allowed starting from the first postoperative day. After the immobilization period, passive and assisted active exercises were initiated for forward flexion and external rotation. After 6 weeks, patients began strengthening exercises of the RC and scapular stabilizers. Rehabilitation was performed with the assistance of physical therapists. Three months after the operation, patients were allowed to practice light sports activity. Heavy manual work and overhead activities were allowed after 6 months.

### Statistical analysis

Statistical analyses were blinded, and performed according to the 'intention-to-treat' principle. The analyses were performed by using SPSS version 16.0.1 (SPSS Inc, Chicago, Illinois). The primary endpoint was the post-operative difference in the Constant score between the 2 groups. The distribution of the Constant score for the 2 groups was normal. Therefore, we used the unpaired T-Test to compare the post-operative results between the 2 groups. 95% confidence intervals were calculated.

The results of each variable of the adopted MRI score were compared with the chi-square test. A significance level of 0.05 was used.

### Power analysis

We performed a pilot study on 20 patients randomised to arthroscopic repair of a RC with or without growth factor augmentation to determine the sample size of participants required to achieve statistical significance in the Constant score at a 0.05 level with 95% power. In the control group, the Constant rating system showed an average of 89 (SD 7.87). In the study group, the Constant rating system showed an average of 93.5 (SD 3.53). Based on these results, a total sample size of 82 participants (41 participants per group) was required to achieve statistical significance at a 0.05 level with 95% power. To compensate for possible loss, we decided to enrol in the study 88 patients. The variable used to calculate sample size was the post-operative Constant score, at 16 months post-surgery. No power analysis was made on the secondary variables.

## RESULTS

No patient experienced infection, neurological or vascular complications.

Detetails of the operated patients are reported in Table 2. Two patients in group 1 and one patient in group 2 experienced a stiff shoulder. They were managed conservatively, with physiotherapy (manual passive motion of the shoulder) for 9 months, when the symptoms resolved.

**Table 2:** Demographics

| | Number of patients | Men/ women | mean age | RC tears size [158] | Tenodesis/ Tenotomy | Number of anchors | Acromioplasty | Infection, neurological or vascular complications |
|---|---|---|---|---|---|---|---|---|
| **Group 1** | 45 | 23 men and 22 women | 55.2 years; range 37 to 69 | small (< 1 cm) in 20, and medium (1-3 cm) in 25 patients | 22/5 | 2 anchors for each patient | 25 | 0 |
| **Group 2** | 43 | 17 men and 26 women | 55.5 years, range 41 to 72 | small (< 1 cm) in 18 patients, and medium (1-3 cm) in 25 patients | 21/3 | 2 anchors for 41 patients and 3 anchors for 2 patients | 12 | 0 |

## Constant Score

*Group 1*

The Constant rating system showed a statistically significant improvement from a preoperative average rating of 42.7 (Std. Dev 7.92) (95% confidence interval [CI], 40.37-45.13) to an average of 88.6 (Std. Dev 7.78) postoperatively (95% confidence interval [CI], 86.32-91) ($P < 0.001$).

We repeated the statistical analysis including only the 38 patients for whom postoperative MRI was available. The Constant rating system showed a statistically significant improvement from a preoperative average rating of 43.42 (Std. Dev 7.7) (95% confidence interval [CI], 40.89 - 45.95) to an average of 89.2 (Std. Dev 8) postoperatively (95% confidence interval [CI], 86.6 - 91.87) ($P < 0.001$). Details of the Constant score are reported in Table 3.

**Table 3:** Constant Score. Average values are given, with the numbers in brackets indicating the range of values.

| | Group 1 (without augmentation with platelet-rich fibrin matrix (PFRM)) | | Group 2 (with PRFM augmentation) | |
|---|---|---|---|---|
| Constant Score | **Pre-operative** | **Post -operative** | **Pre-operative** | Post-operative |
| Shoulder pain | 3.1 (0-5) | 14.3 (10-15) | 3.6 (0-5) | **14.3 (10-15)** |
| Activities of daily living | 10.1 (8-12) | 18.8 (14-20) | 9.8 (6-12) | **19.3 (16-20)** |
| Range of movement | 26.5 (12-39) | 38.8 (26-40) | 26 (16-32) | **39.1 (36-40)** |
| Strength | 3.2 (1-9) | 16.5 (4-25) | 2.6 (0-6) | **15.7 (4-24)** |
| Total Score | **42.9 (22-55)** | **88.4 (54-100)** | **42 (30-53)** | **88.4 (72-99)** |

*Group 2*

The Constant rating system showed a statistically significant improvement from a preoperative average rating of 42.1 (Std. Dev 6.65) (95% confidence interval [CI], 40.06-44.16) to an average of 88.58 (Std. Dev 7.62) postoperatively (95% confidence interval [CI], 86.23-90.92) ($P < 0.001$). Details of the Constant score are reported in Table 1. We repeated the statistical analysis including only the 40 patients for whom post-operative MRI was available. The Constant rating system showed a statistically significant improvement from a preoperative average rating of 42.45 (Std. Dev 6.70) (95% confidence interval [CI], 40.30 - 44.59) to an average of 89 (Std. Dev 7.61) postoperatively (95% confidence interval [CI], 86.56 - 91.43) ($P < 0.001$).

*MRI*

Of 88 participants, MRI results were available for 78 (38 for group 1 patients, and 40 for the group 2 patients). In five patients, there was MRI evidence of a re-rupture: four patients in group 1 (10.5%) and one (2.5%) in group 2 ($P = 0.07$). These patients did not

receive any treatment, as they were satisfied with their clinical conditions. The MRI score was evaluated in the remaining 34 patients in group 1 and 39 patients in group 2 (Table 4).

**Table 4:** MRI score

| | Group 1 (without augmentation with platelet-rich fibrin matrix (PFRM)) | | | Group 2 (with PRFM augmentation) | | |
|---|---|---|---|---|---|---|
| **Variable** | **1** | **2** | **3** | **1** | **2** | **3** |
| **Tendon thickness** | 5 | 12 | 17 | 2 | 10 | 27 |
| **Size of the tendon footprint** | 1 | 10 | 23 | 0 | 4 | 35 |
| **Alterations of signal intensity** | 10 | 21 | 3 | 2 | 13 | 24 |

### Comparison between the 2 groups

There was no statistically significant difference in total Constant Score when comparing the results of arthroscopic repair of the 2 groups (95% confidence interval [CI] −3.43 - 3.9) ($P = 0.44$).

Overall, there was no statistically significant difference in MRI tendon score when comparing arthroscopic repair with or without PFRM ($P = 0.07$).

There was no difference in tendon thickness between the 2 groups as evaluated with the chi-square test ($P = 0.181$).

There was no difference in size of the tendon footprint tendon thickness between the 2 groups as evaluated with the chi- square test ($P = 0.057$).

There was difference in alterations of signal intensity between the 2 groups as evaluated with the chi-square test ($P < 0.01$). The signal intensity was respectively 1.8 in group 1 and 2.6 in group 2.

## DISCUSSION

This randomised controlled trial showed that, in patients with small and medium RC tears, augmentation of the repair with PRFM did not result in significant improved shoulder function (as evaluated with the Constant Score) or structural outcome (as evaluated by MRI) when compared to arthroscopic repair without augmentation of the repair at a minimum 16 months of follow up. There were no serious adverse events related to use of PRFM. At a minimum 16 months of follow up, surgical repair of a RC tear resulted in significant clinical and structural improvement in both groups, independently of the use of augmentation with PRFM.

To our knowledge, this is the first randomized controlled trial to compare the outcome of arthroscopic double row anchor suture repair RC surgery with or without augmentation with PRFM using both clinical and imaging criteria.

We used MRI to evaluate the anatomical appearance following operative RC repair because it allows good definition of the RC and tendon defects, and differentiation of RC degeneration from partial or complete RC tears. Other strengths of this study include the use of a single surgeon, its prospective randomized nature, and the use of independent assessors of the outcome. Also, post-operative management was standardised. A weakness of our study is the absence of information about the number of platelets actually delivered in patients who received the PRFM. Further study is clearly required to evaluate the role of PRFM in rotator cuff repair. Other limitations include the fact that the strength of the shoulder may differ by gender and deteriorate with age, and we did not include this in our statistical analysis because of the relatively small number of patients.

It is difficult to compare the findings of the present study with those of previous reports, as we know of no other prospective studies comparing the clinical and anatomical outcome of small and medium RC tears repaired with or without augmentation with PRFM. In a pilot non-randomized single group study of 14 patients, autologous PRP for arthroscopic RC repair provided good clinical results [521]. A recent randomized controlled trial in patients with chronic Achilles tendinopathy showed no advantages of a PRP injection compared with a saline injection. On the other hand, data from another recent randomized controlled trial showed that treatment of patients with chronic lateral epicondylitis with PRP reduces pain and significantly increases function, exceeding the effect of corticosteroid injection [499]. In a randomised controlled trial, exogenous application of platelet-leukocyte gel during open subacromial decompression contributed to improved patient outcome: recovery was faster and patients returned earlier to daily activities and also took less pain medication than control subjects [179].

RC surgery aims to provide tendon fixation secure enough to hold the repaired tendon in place until biological healing occurs. Several factors may be implicated in failure of RC repairs, including suture or knot failure, inadequate tendon to bone fixation, and lack of tendon to bone healing [95]. As we re-insert tendinous tissue into bone, theoretically only the re-constitution of enthesial fibrocartilage would guarantee an optimal outcome. The concept of autologous platelet-derived growth factors for augmentation of RC repair is appealing, as it should help the re-constitution of enthesial fibrocartilage. However, in the present study, we did not find any beneficial effect of addition of PRFM. Also, the use of autologous platelet-derived growth factors repair results in longer surgical time, is more expensive, and may well be technically more demanding.

The only difference we were able to find between the 2 groups was in alteration of signal intensity. It is difficult to give a clinical significance to this finding. Of concern is that, despite high patient satisfaction rates, healing rates after arthroscopic rotator cuff repair as low as 6% have been reported [211]. A recent systematic review [593] sought to clarify the correlation between structural integrity of the rotator cuff and clinical outcomes. On the basis of the published cohort studies, there were several key differences between healed

and nonhealed repairs in terms of subjective and objective outcomes. Patients with healed rotator cuff repairs after arthroscopic repair can probably expect better strength and possibly better functional outcomes. No definitive conclusion, however, could be drawn because of the variability in the studies (i.e., different outcome scales, strength measurements, and rotator cuff tear characteristics). Furthermore, because the studies were not Level I studies, no metaanalysis could be performed to determine whether a true difference exists between healed and non-intact rotator cuff repairs.

In conclusion, our study does not support the use of autologous platelet-derived growth factors in the form of PRFM for augmentation of a double row repair of a small or medium RC tear to improve the healing of the RC. We did not demonstrate superior clinical or structural performance when compared with the more traditionally, technically less demanding, and economically more advantageous technique of non augmented suture anchor repair. Our results are applicable to small and medium RC tears: it is possible that the use of autologous growth factors contained in platelet rich plasma may be beneficial for large and massive RC tears. Also, given the heterogeneity of platelet rich plasma preparation products available on the market, it is possible that other preparations may be more effective.

# Future and challenges: Instruments to assess patients with rotator cuff tears and animal models for research on rotator cuff

# Chapter 11

## Instruments to assess patients with rotator cuff pathology: a systematic review of measurement properties

## ABSTRACT

**PURPOSE:** The aim of this study was to obtain an overview of the methodological quality of studies on the measurement properties of rotator cuff questionnaires and to describe how well various aspects of the design and statistical analyses of studies on measurement properties are performed.

**METHODS:** A systematic review of published studies on the measurement properties of rotator cuff questionnaires was performed. Two investigators independently rated the quality of the studies using the Consensus-based Standards for the selection of health Measurement Instruments (COSMIN) checklist. This checklist was developed in an international Delphi consensus study.

**RESULTS:** Sixteen studies were included, in which 2 measurement instruments were evaluated, namely the Western Ontario Rotator Cuff Index (WORC) and the Rotator Cuff Quality-of-Life Measure (RC-QOL). The methodological quality of the included studies was adequate on some properties (construct validity, reliability, responsiveness, internal consistency, and translation) but need to be improved on other aspects. The most important methodological aspects that need to be developed are as follows: measurement error, content validity, structural validity, cross-cultural validity, criterion validity, interpretability.

**CONCLUSION:** Considering the importance of adequate measurement properties, it is concluded that, in the field of rotator cuff pathology, there is room for improvement in the methodological quality of studies measurement properties

**LEVEL OF EVIDENCE:** Systematic review of Level-I studies

## INTRODUCTION

Rotator cuff tear (RCT) is a common orthopaedic condition leading to shoulder pain and functional impairment. Despite its frequency and related disability, aetiology and pathogenesis of RCT are still debated. Different measurement instruments have been developed for shoulder pain. Outcomes measures typically fall into two broad categories: general health, joint- and disease- specific. Generic measures are designed to assess functional status regardless of an individual's disease/disorder. Condition-specific measures are designed to be sensitive to the specific disease/disorder of interest. Even though several outcome measures are available for studying patients with impairment of shoulder function, often there is no consensus on which instrument is most suitable for what purpose, as only a few of these instruments have been validated in patients with rotator cuff pathology.

Moreover, not all instruments are developed with use of strict quality criteria [515]. The plethora of available instruments across a broad spectrum of shoulder problems has led to a lack of standardisation in applications, including clinical trials, which has implications for the generalisability of results. High quality instruments provide a useful tool for clinical and research purposes. The quality of some instruments is well documented, but for many it is still unclear [515, 574, 641, 662]. Before considering using or implementing a measurement instrument into a clinical or research setting, one should evaluate its quality [574]. Since the quality of the instrument directly relates to the quality of the studies in which the measurement properties were evaluated, standardized criteria are also needed to assess the quality of these studies [627] Recently, these standardised criteria were published by the COSMIN group. COSMIN stands for Consensus-based Standards for the selection of health Measurement Instruments [445, 446]. According to the COSMIN guidelines, the quality of a measurement instrument is described by three quality domains: reliability, validity and responsiveness. Reliability contains the measurement properties internal consistency, reliability and measurement error, whereas validity contains content validity, construct validity and criterion validity [574].

The aim of this study was to obtain an overview of the methodological quality of studies on the measurement properties of questionnaires for rotator cuff disease and to describe how well various aspects of the design and statistical analyses of studies on measurement properties are performed.

## MATERIALS AND METHODS

### Literature search

A search was performed on June 28, 2011 in MEDLINE (using PubMed 1966–2011), and Embase (using www.embase.com 1974–2011). In PubMed a validated search filter for finding studies on measurement properties was used [628]. The full search strategy is described in Appendix 1. We also performed additional searches with the names of the included instruments (in the title) in combination with the terms for the study population as described in Appendix 1. References of the included articles were reviewed to identify additional eligible articles. The selection of articles was performed independently by three investigators (UGL, AB, and VD). Inclusion criteria were:

- The aim of the study should be to develop or evaluate the measurement properties of an outcome instrument.
- The instrument is used in patients with rotator cuff pathology (as defined by the authors of the included studies) or patients before or after rotator cuff surgery.
- Instruments were included in the review if they were self-assessed, disease-specific (rotator cuff pathology). Furthermore, only studies that were written as full report (that is, no abstract or letter to the editor) were included. We considered publications in all languages. No restrictions were put on the year of publication. Instruments that were developed for groups whose primary complaint did not concern rotator cuff pathology (for example, patients with shoulder instability or glenohumeral osteoarthritis) were excluded.

### Assessment of the methodological quality of the included studies

Two investigators (UGL and AB) independently evaluated the quality of the included studies, using the COSMIN checklist. Disagreements between investigators were resolved by consensus. The COSMIN checklist was developed in an international Delphi study in which consensus was reached on terminology and definitions of measurement properties [446] as well as standards for an adequate study design and statistical analysis of a study on the measurement properties of health-related patient-reported outcomes [445]. This checklist can also be used to evaluate the quality of studies on the measurement properties of other measurement instruments [445].

The COSMIN checklist consists of 12 boxes.

Ten boxes have be used to assess whether a study meets the standards for good methodological quality. Nine of these boxes contain standards for the included measurement properties: internal consistency (box A), reliability (box B), measurement error (box C), content validity (including face validity)(box D), construct validity (i.e. structural validity

(box E), hypotheses testing (box F), and cross-cultural validity (box G), criterion validity (box H), and responsiveness (box I). One box contains standards for studies on interpretability (box J). Each box includes from 4 to 18 items. Each item is scored as "yes", "no", "?" or "not applicable".

In addition, two boxes are included in the checklist that contain general requirements. One box for articles in which Item Response Theory (IRT) methods are applied (IRT box), and one box containing general requirements for the generalisability of the results of a study on one or more measurement properties (Generalisability box).

### Statistical analysis

To assess the agreement between the 2 investigators the Kappa statistic was calculated. The Kappa values were interpreted using the guidelines of Landis and Koch [329]. A Kappa statistic of 0.01 to 0.20 suggests a slight agreement, 0.21 to 0.40 a fair agreement, 0.41 to 0.60 a moderate agreement, 0.61 to 0.80 a substantial agreement, and 0.81 to 1.00 almost a perfect agreement.

## RESULTS

The search strategy identified 731 articles in Pubmed, and 575 in Embase. Evaluation of title and abstract left 119 articles to be evaluated. Full text of all the eligible papers was screened for inclusion and exclusion criteria, leading to 16 studies included in the review [173, 175, 176, 224, 264, 265, 269, 307, 376-378, 385, 450, 491, 524, 671]. The study selection process and reasons for exclusions are summarized in Figure 1.

2 measurement instruments were found for evaluation of patients with rotator cuff pathology, the Western Ontario Rotator Cuff Index (WORC) [307] and the Rotator Cuff Quality-of-Life Measure (RC-QOL) [264]. The WORC [307] was evaluated in 12 studies [173, 175, 176, 224, 265, 307, 376-378, 385, 450, 524, 671], the RC-QOL [264] in 4 studies [264, 269, 491, 524].

The quality of each study is summarised in Table 1, in which each measurement proprieties is scored by an ordinal rating scale.

Construct Validity (or Hypotheses testing) was evaluated in 11 of the 16 included studies. 9 studies evaluated the WORC [175, 176, 224, 265, 307, 377, 378, 450, 524] and 2 the RC-QOL [264, 269]. Only one study on WORC scored as excellent in term of construct validity [175], as all the relevant COSMIN items were scored as adequate. Construct validity was evaluated as good in 3 studies on WORC [224, 307, 377] and 2 studies on RC-QOL [264, 524]. All the other studies ranked as fair in term of construct validity [176, 265, 269, 378, 450], because of small sample size included in the analysis or absence of prior hypotheses to test [176, 265, 269, 450]. In 6 of 10 of the studies [175, 224, 264, 265, 307, 524], hypotheses were formulated a priori. The direction of the

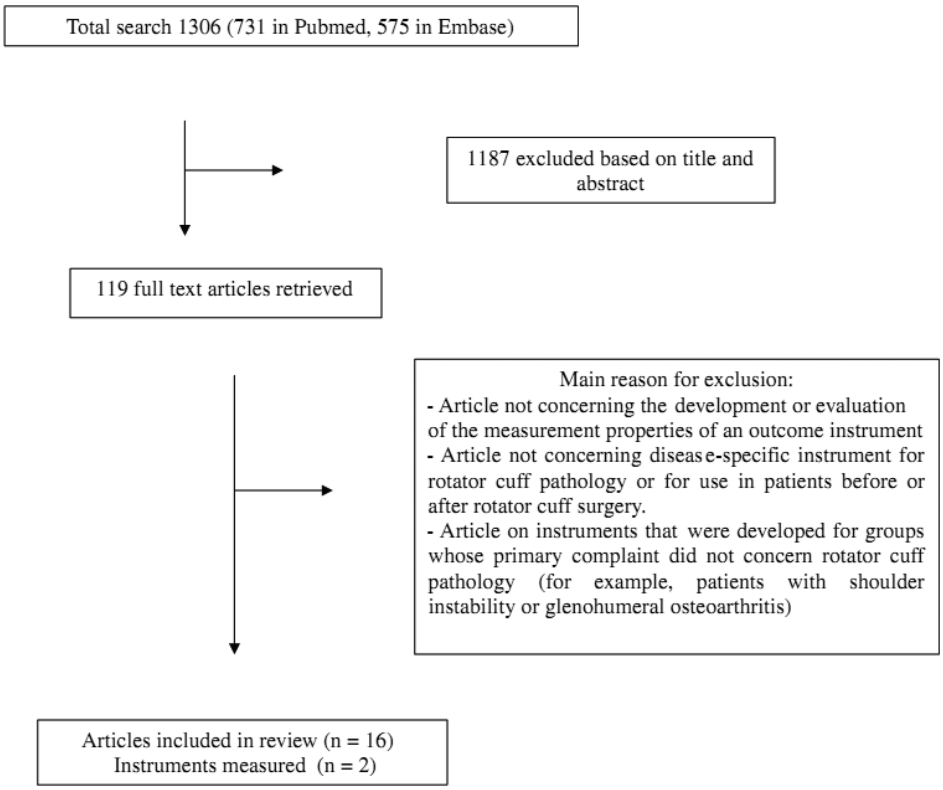


**Figure 1:** Flowchart of the search strategy and selection of articles

expected correlations of differences were quantified in 5 of the studies that formulated hypotheses [175, 224, 265, 307, 524]. The kappa value for interobserver agreement was 0.88.

Reliability was analysed in 8 studies [175, 176, 264, 269, 307, 377, 450, 491]. 5 evaluated the WORC [175, 176, 307, 377, 450], and 3 the RC-QOL [264, 269, 491]. Two articles [175, 377] on reliability on WORC scored as excellent, and two [176, 307] good. The reliability of one study on WORC [450] was considered as low, because it did not describe the percentage of missing items, it had small sample size (45 patients), and time interval between the administrations was inappropriate (48 hours).

Reliability was evaluated as low in all articles on RC-QOL [264, 269, 491], because percentage of missing items was not given, sample size was limited and time interval between the administrations was too short. Reliability was often assessed in relatively small samples. Only 4 of the studies had a sample size of at least 50 patients, which is recommended [175, 269, 307, 377]. The kappa value for interobserver agreement was 0.9.

WORC index was cross-culturally adapted in 3 studies [175, 176, 450], and RC-QOL in 2 studies [269, 491]. WORC was translated into Norwegian [175], Turkish [176] and Persian [450]. RC-QOL was translated into Italian [491] and German [269].

**Table 1:** Methodological quality of the studies on measurement properties (+++: excellent, "++: good, "+: fair, 0: poor. Empty boxes: not applicable. IRT: Item Response Theory. WORC= Western Ontario Rotator Cuff Index, RC-QOL= Rotator Cuff Quality of Life Measure. NA= not applicable)

| Authors (year) | Instrument | Measurement properties assessed | Generalisability per box | IRT used | Score IRT | A internal consistency | B reliability | C measurement error | D content validity | E structural validity | F hypothesis testing | G cross-cultural validity | H criterion validity | I responsiveness | J interpretability |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kirkley 2003 [307] | **WORC** | reliability | "++ | no | | | "++ | | | | | | | | |
| | | hypothesis testing | "++ | | | | | | | | "++ | | | | |
| Wessel 2005 [671] | **WORC** | internal consistency | "+++ | no | | "+++ | | | | | | | | | |
| Hollinshead 2000 [264] | **RC-QOL** | reliability | "+ | no | | | "+ | | | | | | | | |
| | | hypothesis testing | "+++ | | | | | | | | "++ | | | | |
| Ekeberg 2008 [175] | **WORC** | internal consistency | "+++ | no | | "++ | | | | | | | | | |
| | | reliability | "+++ | | | | "+++ | | | | | | | | |
| | | measurement error | "+++ | | | | | "+++ | | | | | | | |
| | | hypothesis testing | "+++ | | | | | | | | "+++ | | | | |
| | | cross-cultural validity | NA | | | | | | | | | "+++ | | | |
| Holtby 2005 [265] | **WORC** | hypothesis testing | "++ | no | | | | | | | "+ | | | | |
| Ekeberg 2010 [173] | **WORC** | responsiveness | "+++ | no | | | | | | | | | | "+++ | |
| Getahun 2000 [224] | **WORC** | hypothesis testing | "+++ | no | | | | | | | "++ | | | | |
| | | criterion validity | "+++ | | | | | | | | | | "++ | | |

**Table 1** (*continued*)

| Authors (year) | Instrument | Measurement properties assessed | Generalisability per box | IRT used | Score IRT | A internal consistency | B reliability | C measurement error | D content validity | E structural validity | F hypothesis testing | G cross-cultural validity | H criterion validity | I responsiveness | J interpretability |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| El 2006 [176] | **WORC** | internal consistency | “+++ | no | | “++ | | | | | | | | | |
| | | reliability | “+++ | | | | “++ | | | | | | | | |
| | | hypothesis testing | “+++ | | | | | | | | “+ | | | | |
| | | cross-cultural validity | NA | | | | | | | | | “++ | | | |
| Lopes 2008 [377] | **WORC** | internal consistency | “+++ | no | | “+ | | | | | | | | | |
| | | reliability | “+++ | | | | “+++ | | | | | | | | |
| | | hypothesis testing | “+++ | | | | | | | | “++ | | | | |
| Lopes 2009 [376] | **WORC** | responsiveness | “++ | no | | | | | | | | | | “+ | |
| Lopes 2009 [378] | **WORC** | hypothesis testing | “++ | no | | | | | | | “+ | | | | |
| MacDermid 2006 [385] | **WORC** | responsiveness | “++ | no | | | | | | | | | | “++ | |
| Mousavi 2009 [450] | **WORC** | reliability | “+++ | no | | | “+ | | | | | | | | |
| | | content validity | “+++ | | | | | | “++ | | | | | | |
| | | hypothesis testing | “+++ | | | | | | | | “+ | | | | |
| | | cross-cultural validity | NA | | | | | | | | | “+++ | | | |

**Table 1** (*continued*)

| Authors (year) | Instrument | Measurement properties assessed | Generalisability per box | IRT used | Score IRT | A internal consistency | B reliability | C measurement error | D content validity | E structural validity | F hypothesis testing | G cross-cultural validity | H criterion validity | I responsiveness | J interpretability |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Papalia 2009 [491] | **RC-QOL** | reliability | "++ | no | | | "+ | | | | | | | | |
| | | cross-cultural validity | NA | | | | | | | | | "++ | | | |
| Razmjou 2006 [524] | **RC-QOL** | hypothesis testing | "+++ | no | | | | | | | "++ | | | | |
| | | responsiveness | "+++ | | | | | | | | | | | "++ | |
| Huber [269] | **RC-QOL** | internal consistency | "+++ | no | | "+ | | | | | | | | | |
| | | reliability | "+++ | | | | "+ | | | | | | | | |
| | | hypothesis testing | "+++ | | | | | | | | "+ | | | | |
| | | cross-cultural validity | | | | | | | | | | "+++ | | | |

No study assessed cross cultural validity. COSMIN checklist was applied only to test translation quality procedure. Translation quality was evaluated as good in 2 studies [176, 491] and excellent in other 3 studies [175, 269, 450]. An adequate procedure contains at least 2 forward and 2 backward translations [90, 320]. This was done in all the studies [175, 176, 269, 450, 491]. The kappa value for interobserver agreement was 1.

Four studies on WORC [175, 176, 377, 671] and one study on RC-QOL [269] evaluated the internal consistency. Quality procedure was excellent in one study [671], good in two studies [175, 176] and fair in two studies [269, 377]. In all the studies, Cronbach α was calculated [175, 176, 269, 377, 671]. This is an adequate parameter of internal consistency. The sample size was usually adequate (100%). In 4 of the 5 studies, unidimensionality of the scale was not checked [175, 176, 269, 377]. 11 of 15 studies did not evaluated the internal consistency [173, 224, 264, 265, 307, 376, 378, 385, 450, 491, 524].

The kappa value for interobserver agreement was 0.85.

Only one study [450] analysed content validity of WORC. The quality of this assessment was good. It assessed items relevance for target population and purpose of the measurement instrument and their comprehensiveness. More emphasis could be placed on examining whether the items are relevant for the construct (eg, by asking experts or patients). 14 of 15 studies did not evaluate the content validity [173, 175, 176, 224, 264, 265, 307, 376-378, 385, 491, 524, 671].

The kappa value for interobserver agreement was 1.

Responsiveness was evaluated in 4 studies, 3 on WORC [173, 376, 385] and 1 on RC-QOL [524]. One study was scored as excellent [173], as it met all COSMIN requirements. Two studied were scored as good [385, 524]. One did not formulate a priori hypotheses about changes in scores [385]. The other did not clearly describe time interval between measurements, what happened in the interim period, and change in at least a percentage of patients [524]. One study [376] was considered of poor quality because of the small sample size included in the analysis (30 patients), lack of a priori hypothesis and lack of adequate description of the comparator instruments. 11 of 15 studies did not evaluate the responsiveness [173, 175, 176, 224, 264, 265, 307, 377, 378, 450, 491, 671].

The kappa value for interobserver agreement was 1.

Measurement error was evaluated only in 1 study [175] on WORC. The quality of its assessment is excellent. It met all design requirements. Measurement error was expressed by limits of agreement.

The kappa value for interobserver agreement was 1.

Only one study analyzed criterion validity [224]. It is on WORC and its assessment of criterion validity is of good quality. The kappa value for interobserver agreement was 1.

Structural validity was not evaluate in any of the included studies.

None of the included studies analysed interpretability.

Generalisability box has been completed several times, for each property investigated in each study.

Generalisability of 9 studies on 20 proprieties was excellent, and generalisability of 6 studies on 7 properties of was good. Only generalisability of reliability of one study [264] was scored as fair, because characteristic of patient sample used for reliability evaluation are not clearly described. The kappa value for interobserver agreement was 1.

## DISCUSSION

The most important finding of the present study was that even though several studies are available to evaluate shoulder disease, they generally assess all conditions affecting the shoulder, instead of disease-specific outcome measures that are designed to assess specific conditions in individual joints, such as rotator cuff pathology. Only 16 articles encountered the inclusion criteria to be included in the present systematic review on rotator cuff specific instruments Several studies were excluded because they did not investigate measurement proprieties of outcome instruments.

The methodological quality of the included studies was assessed by the COSMIN list, which was recently developed by a multidisciplinary, international consensus-study [445, 446]. Experts in health status measurements from all over the world developed standards for methodological quality of studies on measurements proprieties [445, 446]. No others checklist are available for this purpose.

Evaluation of methodological quality of available studies on measurements proprieties is essential to understand the appropriateness of conclusion about an instrument and to guide further researches on measurement properties. To our knowledge, this is the first study that systematically evaluated the methodological quality of studies on measurement properties in rotator cuff pathology.

The methodological quality of the 15 studies varied widely.

Construct validity is the degree to which scores of an instrument are consistent with hypotheses based on the assumption that the instrument validly measures the construct to be measured [445, 446]. Hypotheses may concern expected mean difference between groups or expected correlations between the scores on the instrument and other variables [25, 27]. Construct validity was often analyzed in the paper included in the present systematic review. Studies on construct validity were generally well performed. Many, but not all the studies, formulated hypotheses before the data collection, thus preventing from the risk of bias in interpretation of the results [445, 446]. The direction of the expected correlations of differences was frequently expressed, while the magnitude was rarely quantified. These considerations are important to decide afterword whether the hypothesis is confirmed or not.

It is also important to describe comparator instruments and their measurement properties, in order to discriminate between poor validity of the instrument under study or

poor quality of the comparator instrument. Generally, the studies included in this review adequately described comparator instruments but they provided only the reference of studies on comparator instruments properties. It would be more helpful if a short summary of the measurement properties would be presented.

Studies on rotator cuff instruments often analysed reliability and their methodology was generally adequate [445,446]. Reliability represents the total variance in the measurement which is due to "true" difference between patients [574]. Basic requirements are at least two measurements available in similar conditions with adequate time interval. Generally, in the studies included in the present review, instruments were administered twice, but test conditions were specified only in some cases. Moreover, not always time interval was long enough to prevent recall bias, and short enough to ensure that patients have not been change on construct to be measured [445,446]. A time interval of about 2 weeks is advised. Another issue of concern was definition of "stable patient". It is recommended an assessment of a global rating of change, completed by the patient or the physician. Statistical analyses for assessing reliability were often well performed. However, several studies had relatively small sample sizes, with less than the 50 patients recommended.

At present, translation studies of specific rotator cuff instruments are not numerous, but their methodology was usually adequate [574]. They based on several existing guidelines for translation and adaptation of measurement instruments [90,320,445,446]. They performed multiple forward and backward translations with at least two independent translators whose qualifications were described. The final translation was reviewed by a multidisciplinary committee. The methodological aspects that need to be improved is the performance of a pretest to check interpretation, comprehension, and cultural relevance of the items. It was done only in part of the studies included in this review.

No study assessed cross cultural validity. It defines the degree to which the performance of the items on a translated or culturally adapted instrument is an adequate reflection of the performance of the items of the original version of the instrument.

Internal consistency is the degree of the interrelatedness among the items[445,446]. In the studies included in this review internal consistency was often not assessed, but if assessed, the methodology was usually adequate [574]. On one point the methodological quality can be improved. Few studies checked the unidimensionality of the scale [445,446]. It is needed to give an interpretable meaning to internal consistency statistic. Moreover, Cronbach α should be calculated for each sub-scale separately.

Content validity is the degree to which the content of an instrument is an adequate reflection of the construct to be measured [445,446]. It is a very important measurement property, but the literature lacks of studies on the subject. It should be assessed by making a judgment about the relevance and the comprehensiveness of the items [574]. Moreover all items should be relevant for the study population. These questions should be examined respectively by a group experts and the target population.

Responsiveness is the ability of an instrument to detect change over time in the construct to be measured [445, 446]. Studies that analyzed responsiveness were well performed. Standards for responsiveness are similar to those of construct validity [574]. The only difference between validity and responsiveness is that validity refers to the validity of a single score and responsiveness refers to the validity of a change score [445, 446].

Hypotheses were formulated less frequently in responsiveness studies than in validity studies. To evaluate responsiveness at least two measurements are necessary and it is essential that at least a part of patients changes. What happened in the interim period should be described. These requirements are generally provided by the studies. Time interval between measurements should be stated. However, according to the COSMIN guidelines, there are not indications on how long it should be. Description of what happened in the interim period is essential as it is more important than the actual time period

Responsiveness studies should use adequate statistical methods, such as correlations and ROC curves. P values and effect sizes should not be used because they are not relevant to examine whether statistically correlations differ from zero.

Measurement error assess the systematic and random error of a patient's score that is not attributed to true changes in the construct to be measured [445, 446]. It requires the same study design and data used for reliability [152]. However, studies on reliability did not exploit the data collected to analyze measurement error. It is desirable that measurement error integrates information of reliability. Requirements are: adequate sample size, at least two measurements, adequate time interval, stable patients, and similar test conditions [574]. The preferred statistic is Standard Error of Measurement (SEM), Smallest Detectable Change (SDC) or Limits of Agreement (LoA). It is recommended that parameters of measurement error are calculated in reliability studies.

Criterion validity represents the degree to which the scores of an instrument are an adequate reflection of a gold standard [445, 446]. Studies on criterion validity of rotator cuff instruments are missing [574]. The main difficulty in assessing this property is that no gold standard exist for health–related questionnaires. The only exception is instrument's original long version compared with shortening version. Authors can erroneously consider comparator instruments as a gold standard, thus the criterion used should be reasonable [445, 446]. Correlation is the preferred statistical method when both the instrument and the gold standard are continuous scores, the area under the receiver operating characteristic is the preferred method when instrument score is continuous and gold standard score is dichotomous, sensitivity and specificity are preferred methods when both instrument and gold standard scores are dichotomous.

It is recommended that more studies are performed on criterion validity.

Structural validity is the degree to which the scores of an instrument are an adequate reflection of the dimensionality of the construct to be measured [445, 446]. Literature is completely devoid of studies on structural validity [574]. Studies on instrument based on a

reflective model should consider this property. The structure of the instrument should be assessed with factor analysis. The dimensionality of the items should be assessed by IRT tests.

It is recommended that more studies are performed on structural validity.

Interpretability is not considered a measurement property, but an important characteristic of a measurement instrument [445,446]. It represents the degree to which a qualitative meaning, clinical or commonly understood connotations, can be assigned to an instrument's quantitative scores or change in scores [445,446]. It has a relevant implication in research or clinical practice. Studies on interpretability of rotator cuff instruments are needed.

Adequate description of patients sample is useful to understand to which population the result of a study can be generalized [574]. Studies usually completely supply sample characteristic. Overall, the generalisability box scored better than the quality of the assessment of the properties for each article [445,446].

A strength of this review was the use of a standardized procedure and the COSMIN checklist to assess the quality of manuscripts. Moreover, the quality of each article was assessed by 2 independent investigators, as recommended by the COSMIN group [445,446]. The investigators had a perfect agreement as reflected in a Kappa score between 0.85 and 1 [329].

However, for several items of the COSMIN checklist, a subjective judgement is needed, which means that other investigators may have rated differently some aspects. Furthermore, it was not possible to discriminate between poor quality and poor reporting, and, therefore, it was not always clear whether certain design aspects were not performed or not reported and this may have affected the quality ratings.

A limitation of this review is that we did not contact authors for further information if things were unclear or not reported. This may have influenced our ratings of the quality and results of the studies to some extent. However, the data were extracted independently by two investigators. Also, another limitation of this systematic review was that only 16 studies encountered the inclusion criteria, because of the lack of well performed studies on the topic. There is therefore, an urgent need for studies on the topic that use the COSMIN checklist to evaluate the methodological quality of study protocol.

The clinical relevance of this systematic review is to have elucidated the quality of clinimetric properties of available scores for rotator cuff pathology, by using common checklists such as COSMIN. In designing a study protocol, it is important to formulate the research question and then apply the appropriate instrument that addresses the aims of the study. Rotator cuff measures such as the WORC and RC-QOL should be used to evaluate outcome of patients with rotator cuff disease.

As the quality of validation studies continues to increase then the patient-oriented instruments can be more carefully selected. Subsequently, the future orthopaedic care of patients with rotator cuff disease may also improve.

## CONCLUSIONS

Rotator cuff tears are frequent and cause elevate sanitary costs in industrialised countries. Aetiology is still largely unknown and surgical strategies need to be optimised. Evaluation of outcomes is extremely important when the outcome being measured is subjective, as in the assessment of rotator cuff pathology. In this field, there is room for improvement in the methodological quality of studies on measurement properties. Before developing new instruments to assess rotator cuff pathology, it is important to better describe measurement properties of the available questionnaires to point out the needed modifications.

# Chapter 12

## Animal models for translational research on shoulder pathologies: from bench to bedside

## ABSTRACT

Several animal models have been used for *in vivo* and *in vitro* shoulder research. *In vitro* models, consisting of cadaveric specimens, are useful in providing basic understanding of the functioning of the shoulder and for biomechanical experiments. *In vivo* models provide the means to model living phenomena, such as tendon healing process, tendinopathy, instability and adaptive responses to surgery. However, intrinsic differences among different species make translation to human shoulder pathologies difficult.Most of the animals used in experimental settings are quadrupeds, using the forelimbs for weight-bearing during locomotion, with no or minimal overhead activity. The various animal models already used to study shoulder pathologies are presented in this article. However, there is a lack of validation for these animal models, which provides challenge to the further research in this field.

## INTRODUCTION

The use of animal models to study human pathology is valuable in many fields. Animal models of disease have successfully and accurately reproduced many aspects of human illness allowing for indepth study of pathophysiology. These models have been the source of much information, including the importance of certain molecular mechanisms and genetic contributions in several diseases.

Numerous animal models have been used for *in vivo* and *in vitro* shoulder research, such mouse and rat [167, 229, 285, 438, 439, 503-506, 559, 560, 600, 601, 682], cats [178], rabbit [180, 205, 314, 318, 319, 420, 441, 462, 484, 557, 640], goat [183, 206, 387, 608], sheep [83, 128, 220, 221, 435, 464, 566, 603], dog [6, 30, 31, 156, 160, 163, 277, 306, 470, 551], calf [71, 316, 410], cynomolgus monkey [195], baboons [597]. *In vitro* models, consisting of cadaveric specimens are useful in providing basic understanding of the functioning of the shoulder [62, 63]. *In vivo* models provide the means to model phenomena, such as tendon healing process, tendon degeneration, instability and adaptive responses to surgery. Basically, human specimens are more suitable for these models than are animal specimens whenever anatomy, size and kinematics are important. However, there are some disadvantages in using the human model. One problem is the difficulty in obtaining fresh human specimens, especially from younger subjects. These disadvantages of human specimens force a search for alternative animal models [161].

The presence of a validated animal model would enable in-depth studies on the aetiology, molecular mechanisms and potential treatments of different shoulder pathologies, as animals are more homogeneous and are easier to control than humans [381]. As we can better control the variation in animal experiments, it would be possible to isolate the effect of a single factor [381]. Tissue specimens can be obtained easily and at early time points before the onset of symptoms, which is not possible in humans [381]. The availability of preclinical data is definitely essential before the potential treatment modalities are studied in clinical trials according to the Food and Drug Administration requirements [381]. Therefore, animal models are indispensable for shoulder research. However, the use of animals for experimentation also raises many concerns about the welfare of the animals [381]. To make both scientific and animal welfare decision, experiments must have the clear objective of improving the welfare of man and/or animals, and the researchers need to keep constantly animal welfare at the forefront to ensure humane treatment of all animals [381]. It is necessary to carefully study the possible behavioral changes of the animal such as pain, stress and discomfort, which may be related to the scientific interests at hand, but more to the animals' welfare. The principles of three Rs (replacement, reduction and refinement) in animal studies should be observed [61]

Animal models help to understand the natural history of various diseases and conditions, and provide a means by which the effectiveness of different therapeutic interventions can be assessed. However, given the differences among different species, it is sometimes

difficult to reproduce reliable diseases phenotype in animals. While each of these animal species may possess bony and soft-tissue anatomy with varying similarities to the human shoulder, none is the same [161]. Each of them has peculiar advantages or disadvantages. An ideal animal shoulder model should have similar anatomy and function as human, an intrasynovial injury environment, possibility to develop a chronic injury condition, tendon size similar to human (to allow for standard techniques of repair), muscle atrophy, stiffening, and fatty infiltration after a tendon tear, absence of spontaneous tendon healing or scar formation without treatment, incidence of tendon re-tear, the ability to control postoperative mechanical loading on the repair [161]. There are obvious stark marked anatomical differences between quadripeds and bipeds, especially in the forelimbs. Most of the animals used in experimental settings are quadrupeds, using the forelimbs for weight-bearing during locomotion, with no or minimal overhead activity. Also, differences exist between absolute quadrupeds (e.g. goat, sheep, calf) and quadrupeds also working with their hands standing on their legs (e.g. rat, squirrels, monkey). Quadrupeds use its supraspinatus to accelerate a pendulum, while in humans it raises their arm and acts at a disadvantage against gravity and under great strain [598]. While movement of quadrupedal shoulders is largely restricted to the sagittal plane, those of bipedal primates can additionally rotate and move in the coronal plane, thereby allowing much more mobility [598]. From an evolutionary point of view, this necessitates adaptations in the architecture of the bone and soft tissue [598].

The absence of validated animal models for the study of shoulder pathology challenges the research on this field. To further our understanding of shoulder pathology, suitable animal models are required. This paper overviews the role of animal models in shoulder research.

## ANIMAL MODELS

### Rat Model

One of the most commonly used animal model in shoulder pathology is the murine model [439]. Soslowsky et al [600] developed a rat model which is regarded as having the greatest similarity to human shoulder with respect to bony anatomy and activity (overhead reaching). In the seminal Soslowsky' work [600], 36 rats were randomized to three experimental groups. One group (n = 12) underwent an intratendinous injection of bacterial collagenase simulating an acute intrinsic injury, another group (n = 12) underwent an acromial alteration to reduce the subacromial space simulating an external compression, and the third group (n = 12) underwent a combination of both interventions. Significant increases in cellularity, number of fibroblasts, and collagen disorganization were seen in all experimental

tendons compared with a contralateral control group, supporting the rat as an appropriate model for investigating rotator cuff disease. Unlike many other animals, the rat shoulder has a coracoacromial arch and is most similar to the human shoulder [161]. The rat acromion directs anteriorly over the humeral head to the clavicle, determining an enclosed arch over the supraspinatus [600]. Similarly to the human shoulder, excursion of the supraspinatus occurs immediately below the acromial arch when rat walks, burrow, and reach overhead (such as for food) [600]. Therefore, the rat model has been particularly useful to study the mechanisms of supraspinatus tendon injury involved in the pathogenesis of rotator cuff disease, especially those processes related to extrinsic tendon damage caused by repetitive motion injuries (treadmill running) or impingement [600]. However, Schneeberger et al [570] questioned this model, as the portion of the rat supraspinatus muscle that passes under the acromial arch is muscular, and not tendinous as it is in humans.

Rats have been also used to study the gene expression [285], mechanisms [214, 634, 682], healing [125, 215, 231, 636], and regenerative strategies [166, 240, 453, 635] for acute tendon-to-bone repair. This animal model has been used previously to study the role of intrinsic injury modeled such as an acute insult to the tendon [104, 600], extrinsic injury modeled as external subacromial impingement [104, 600] and overuse factors [602] on rotator cuff tendinopathy. These studies showed that it is possible to produce rotator cuff tendinopathy via isolated intrinsic, extrinsic, or overuse injury [601].

Importantly, re-tear after rotator cuff repairs in rats has not been observed postoperatively [212]. This is an important difference with the behaviour of human rotator cuff repair, in whom retears occur, and make the rat a less suitable model for evaluating repair strategies that are engineered to target the critical need for mechanical efficacy in human rotator cuff repair. Recently, the rat has also been used to study the use of scaffold devices for rotator cuff repair augmentation [126] or interposition grafting across a large rotator cuff defect [275, 507, 690].

As it is possible to control postoperative loading on the tendon-to-bone repair in rat, this model has been used to evaluate the effect of postoperative activity levels (muscle paralysis, free cage activity, casting, and exercise) on acute tendon-to-bone healing [212, 231, 636].

Furthermore, the rat well tolerates bilateral shoulder surgery [126, 275, 634], which offers the experimental advantage of having a paired control.

The rat model has been also used to investigate chronic rotator cuff repair [59, 213, 228-230, 346]. The rat model also has the advantage of the availability of rat-specific molecular and immunohistochemical reagents. The homology between humans and rodents is reflected genomically. Comparative mammalian genomics has indicated that as many as 80–90% of rodent genes have matches in humans [669], enabling rodents to be similar enough to humans to provide useful translational experimental data [1]. Peculiar advantages of rats and mice (rodents) over other species include the short gestation periods, delivery of multiple offspring and the rapid growth rates and short life spans, enabling studies to be

performed efficiently [669]. Moreover, their mild temperaments enable ease of handling, and are relatively inexpensive as they are readily available and require low maintenance [669]. Rats are reportedly easier to work with than mice as they are less aggressive and readily trained [1, 669].

The main limitation of rats compared to mice is the current limited number of transgenic strains, for it has proven more difficult to obtain transgenic rats compared to mice [669]. Another obvious disadvantage of the murine model is the small size, making it impossible to evaluate many implants and making surgical procedures tedious and tissues delicate to handle [537]. Moreover, the tendon of the rat rotator cuff differs from humans because they are aligned and not interdigitated. They appear to be confluent with the underlying joint capsule only at their insertions [161].

Barton et al [59] recognized a lack of irreversible muscle fat accumulation in the surgically produced chronic rotator cuff tear in rats, which again contrasts with the human condition [232].

Remarkably, like other animal models, the rat undergoes scar tissue formation and healing of the rotator cuff injury in the absence of treatment [275, 690] which limits the ability to discriminate nonefficacious treatments for the human condition, where spontaneous healing does not occur [161].

**Rabbit**

The rabbit is frequently used in orthopaedic research. Rabbits have the advantage of having larger tendons when compared to rats, which provide larger samples for analysis and are easier to manipulate during surgical operation [381]. However, they are less tough compared with rats, and can die easily after surgery or from diarrhea [381]. Special care needs to be provided to rabbits used as the animal model [381]. A further disadvantage is their susceptibility to serious, life-threatening injury (fracture/dislocation at the lumbosacral junction) when suddenly frightened [669]. While rabbits are popular animal models, better understanding about their behaviour and physiology is essential as these animals may use different mechanisms for balancing, locomotion and pain perception compared with human [381].

This model has been used to evaluate the effect of a periosteal flap taken from the proximal tibia on the healing of the infraspinatus tendon and bone [109].

Almost all rabbit rotator cuff studies have been conducted on the supraspinatus tendon [242], the most commonly injured tendon in humans. However, the supraspinatus and infraspinatus tendons are significantly different between rabbits and humans. The acromion of the rabbit scapula is a relatively rudimentary structure (Figure 1), forming an arch that the infraspinatus and teres minor pass underneath, with a muscular rather than tendinous portion in contraposition with the human rotator cuff [242]. The anterior

**Figure 1:** 3-D computed tomography reconstruction of a right rabbit shoulder, showing the prominent greater tuberosity, prominent upper portion of the glenoid fossa and absence of the acromion.

aspect of the glenohumeral joint contains an additional bony tunnel with its boundaries being the tuberculum supraglenoidale laterally, the coracoid process superiorly, the tuberculum infraglenoidale inferiorly, and the coracobrachialis muscle medially [242]. The rabbit subscapularis tendon passes under the tuberculum supraglenoidale and inserts on the lesser tubercle of the humerus in an analogous manner to the human supraspinatus tendon passing under the acromion to the greater tuberosity [242]. Grumet et al [238] proposed the rabbit subscapularis muscle to be an accurate and reliable animal model for the study of rotator cuff tendinopathy, identifying a relationship of the rabbit subscapularis tendon and scapular bony tunnel.

The earliest studies on rabbit rotator cuff models focused on the secondary response of the affected muscle after tendon injury. The rotator cuff tendon was detached and muscle atrophy, twitch tension, fatigue index, and discharge of the mechanosensitive afferent units were analyzed [72, 73, 180-182]. These studies evidenced marked fatty infiltration of the supraspinatus muscle after tendon nerve transection. These findings were confirmed also in the subscapularis tendon of rabbit: fatty infiltration of the subscapularis muscle was significantly greater in rabbits with complete tendon injury and nerve transection [242].

In the current literature, there is little experience with a rabbit model for shoulder pathologies, and further evaluation has to be performed

### Sheep - Goat

Skeletally mature female sheep, and to a lesser extent goats, have several advantages that are increasing their use in experimental settings [644]. They represent a convenient large-

animal model because of ease of handling and housing availability, cost, and acceptance to society as a research animal [128,537,644].

The size of the infraspinatus tendon of sheep allows it to be particularly useful for biomechanical studies [62,63,83,143,384]. Although the anatomy of shoulders of quadruped animals is different from humans, sheep have been chosen because of the similarity of the infraspinatus tendon to the human supraspinatus tendon. The human supraspinatus tendon is intraarticular, while in the sheep it is extraarticular. However the sheep infraspiantus tendon lies on a bursa, guaranteeing some contact of the repair with a small amount of synovial fluid, even though the ovine bursa likely does not have the volume of synovial fluid contained in the human shoulder joint [644]. The histopathological, biochemical, and biomechanical processes of rotator cuff repair have been studied following tenotomy of the infraspinatus of goats [608] and sheep [221], and subsequent reattachment to the proximal humerus. However, one of the major shortcomings of the use of the sheep supraspinatus tendon is the frequent finding of robust scar formation between the retracted tendon and the bone, while failure to heal occurs in most cases in human rotator cuff tears [644]. Even though the acute tenotomy and reattachment to the proximal humerus is not a faithful reproduction of human clinical conditions, it represents an interesting model to test different treatment options (suture anchors, scaffolds [464,565], growth factors [464], low intensity pulsed ultrasound [466]).

The use of sheep rotator cuff as chronic lesion model is complex [161,644]. Animals heal rapidly, with a large amount of fibrous tissue formation and neovascularization [644]. Delayed repair of the detached infraspinatus tendon was not recommended because of the difficulty in distinguishing scar tissue from normal tendon at the time of reattachment.

The end of the infraspinatus tendon was wrapped with Gore-Tex (Preclude; W. L. Gore & Associates, Flagstaff, AZ) to reproduce a chronic rotator cuff injury [128]. Wrapping the end of the tendon at the time of the initial surgery may allow to clearly identify the cut tendon during the second procedure and, consequently, to perform a secure repair [128]. An osteotomy of the greater tuberosity of the humerus can be useful to aid to identify the detached infraspinatus tendon after long periods of detachment [220].

The chronic model of tendon detachment provides the surgeon a better understanding of the timing of the repair and the temporal aspects of healing [128,220,434]. Tuner et al [644] proposed that a long-standing chronic model (detached for periods greater than 8 weeks) to be more useful to evaluate bioimplants (eg, collagen scaffolds) rather than attempted reattachment to the bone, recommending infraspinatus detachment and covering, and then reattachment, as soon as 4 weeks if bone-to-tendon healing is to be evaluated. Surgery to reattach the tendon can be scheduled 8 weeks after the detachment and covering surgery [644], if the ability of a bioimplant, scaffold, autologous platelet-rich fibrin matrix, a growth factor or a combination of these to bridge a large gap is to be evaluated.

One of the problems encountered with experimental surgery in sheep is related to the difficulties in protecting the repair from full weight bearing postoperatively and controlling the postoperative loads on the repaired tendon [644]. A rubber ball was positioned under the hoof of the operated limb and removed at 5 weeks to evaluate whether this determined a restricted limb movement, but the results were dubious [338]. Turner et al [644] reverted to close-stall confinement postoperatively. Although several of these repairs fail to heal by tendon-to-bone healing, the model can be still useful to evaluate the effect of chronic tendon detachment on muscle atrophy and fatty infiltration [644]

The sheep shoulder has been also used in the setting of experimental glenohumeral instability [466]. However, given the different orientation of the scapula and tuberosities, the different size of tuberosities when compared to human shoulder, we do not recommend their use for experimental glenohumeral instability studies (Figure 2-4).

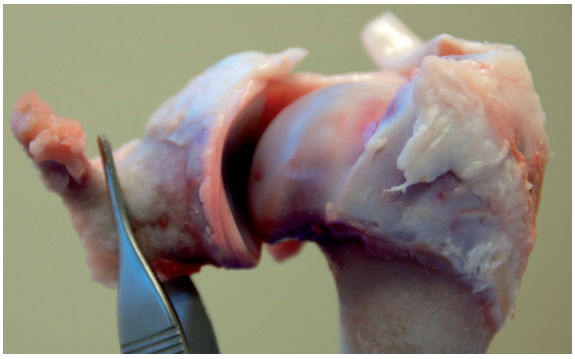

**Figure 2:** Macroscopic picture of the left shoulder in a sheep, showing the elongated humeral head, the deep glenoid and the prominent greater tuberosity.

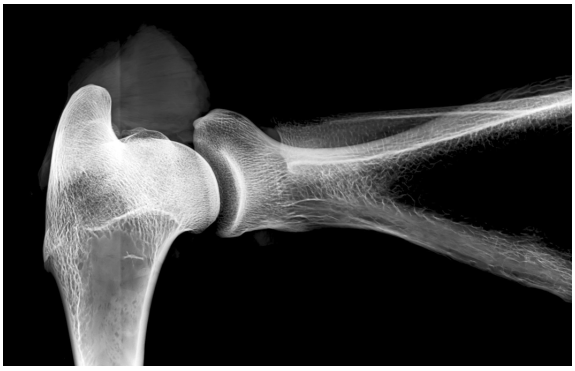

**Figure 3:** Radiograph of the left shoulder in a sheep, showing the elongated humeral head, the deep glenoid and the prominent greater tuberosity

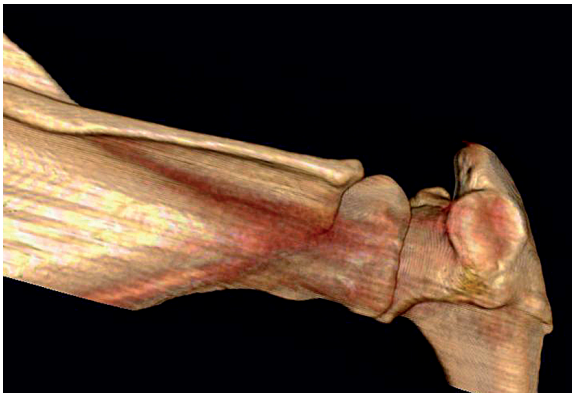

**Figure 4:** 3-D computed tomography reconstruction of the left shoulder in a sheep, showing the prominent lesser and greater tuberosities

## Dog

The canine shoulder has been used to evaluate different rehabilitation modalities, including slinging, hobbles, casting, walking through obstacles, swimming, jumping down from graduated heights, exercise bands, and treadmill walking or running in air or underwater [161,416]. Derwin et al [160] evaluated the utility of the canine model for studies of acute, full-thickness rotator cuff tendon injury and repair. They found that time-zero failure load is dependent on the suture type and configuration used for repair. Acute, full-width tendon repairs fail anatomically within the first days after surgery in the canine model, regardless of suture type, suture configuration, or postoperative protocol. Robust scar tissue forms in the gap between the failed tendon end and the humerus, which can be visually, mechanically, and histologically misconstrued as tendon if an objective test of repair connectivity is not performed. The authors [160] concluded that a full-width injury and repair model in the canine will provide a rigorous test of whether a new repair strategy or postoperative protocol, such as casting or temporary muscle paralysis, can maintain repair integrity in a high-load environment. Alternatively, a partial-width tendon injury model allows loads to be shared between the tendon repair and the remaining intact portion of the infraspinatus tendon and prohibits complete tendon retraction. Thus, a partial-width injury in the canine may model the mechanical environment of many single tendon tears in the human injury condition and warrants further investigation. Dog also mimics the human condition in that muscle stiffness increases and atrophy and fat accumulation occurs and persists in chronically detached muscles [160,551]. Safran et al [551] developed a chronic rotator cuff tear in a canine model to investigate and quantify the time-related changes in passive mechanics, volume, and fat of the infraspinatus muscle. They surgically detached the right infraspinatus tendon of eight adult mongrel dogs from the proximal part of the humerus. The uninvolved left shoulder served as a control. Muscle volume changes were

quantified with use of magnetic resonance imaging. Intramuscular fat was evaluated histologically at the time that the animals were killed. After twelve weeks of detachment, the stiffness was significantly increased in the detached infraspinatus muscles relative to that in the controls. Magnetic resonance image analysis demonstrated that the detached muscle volumes decreased by an average of 32% in the first six weeks and remained constant thereafter. Intramuscular fat increased significantly in the detached muscles and to a greater extent in the lateral regions [551]. The canine shoulder has been used for rotator cuff repair augmentation with use of a woven poly-L-lactide device [163], in arthroplasty experiments to evaluate the healing of reamed glenoid bone articulating with a metal humeral hemiarthroplasty [419], to develop a method of tendon attachment to a metallic endoprosthesis [235], and to evaluate biologic tendon fixation to metallic implant augmented with autogenous cancellous bone graft and bone marrow [277]. Radiographical, mechanical, and histologic evaluation of 2 glenoid prosthesis designs was also evaluated in a canine model [677]. Biomechanical tests have been performed as well on canine shoulder [425, 586]. The most evident difference of bony architecture of shoulder in dogs in respect to the human one are the flattened and elongated humeral head, the prominent tuberosities and the deep glenoid (Figure 5-7).

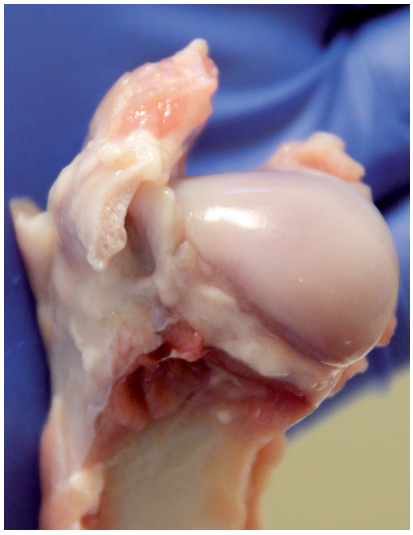

**Figure 5:** Macroscopic picture of the left shoulder in a dog, showing the flattened humeral head, and the prominent greater tuberosity.

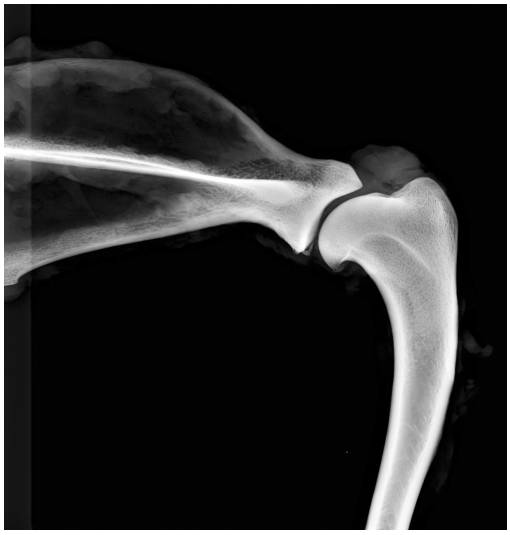

**Figure 6:** Radiograph of the left shoulder in a dog, showing the elongated humeral head, the deep glenoid and the prominent greater tuberosity

**Figure 7:** 3-D computed tomography reconstruction of the left shoulder in a dog, showing the prominent greater tuberosity

## Calf

Calves have been used mainly for biomechanical experiments [71, 316, 410]. A major advantage of bovine shoulder is the consistency of rotator cuff dimensions and tissue quality, which helps from the perspective of experimental consistency. Using this particular model in biomechanical setting, the common failure modes of anchor pullout and suture cutting through tendon are eliminated [410].

## Non-human primates

From a translational point of view, non-human primates are the most ideal species to use in experimental shoulder setting, as they are the closest to humans in terms of anatomy and physiology. Obviously, ethical and economic concerns limit their use in experimental settings [669]. Recently, Sonnabend et al described the anatomy of the rotator cuff in 22 different animal species [598]. In most of the species studied, the tendons of supraspinatus, infraspinatus and teres minor were inserted independently into the greater tuberosities of the humerus, with no intertendinous connection. Thus, these animals did not possess a true rotator cuff. A true rotator cuff was found only in advanced primates, and in one unusual species, the tree kangaroo [598]. The presence of a true rotator cuff appeared to be associated with the ability to carry out regular overhead activity and to use the upper limb away from the sagittal plane [598]. The baboon is the best animal model to study the repair of the rotator cuff because of the similarity of the shoulder to that of man. To our knowledge, the only reported study to date of repairs of the rotator cuff in a primate model was performed by Sonnabend et al [597]. Healing of the baboon supraspinatus involved a sequence of stages resulting in the reestablishment of the bone-tendon junction. Although macroscopically the repair appeared to be healed at eight weeks, the Sharpey fibres holding the

repair together did not appear in any considerable number before 12 weeks. By 15 weeks, the bone-tendon junction was almost, but not quite mature. These results support the use of a post-operative rehabilitation programme in man which protects the surgical repair for at least 12 to 15 weeks to allow maturation of tendon-to-bone healing [597].

**Evolution of shoulder anatomy**

During the evolution of the upper extremity, the scapula, more than any other bone of the shoulder girdle, reflects momentous alterations that have been determined by increased functional demands of a prehensile limb [159]. Changes in posture provided the stimulus which initiated the numerous morphologic changes [159]. With increased efficiency in locomotion, there was a trend toward reduction of this bone, the glenoid cavity shifting from a position directed laterally to one directed posteriorly and inferiorly. As a result of the change in posture, the function of the coracoid decreased [159]. Posture was responsible for the development of the scapular spine [159]. The shape of the scapula is dependent upon posture and the functional requirements of the muscles attached to it [159].

There is a tendency for a relative increase in the size of the infraspinous fossa with progression from quadrupedal primates, through arboreal (tree-living) to bipedal species [276, 598]. A progressive distal migration of the point of insertion of the deltoid was observed, together with the increasing size of the acromion, and noted that these changes increased the functional advantage of the deltoid [276, 598].

Other morphological changes described during the evolution of the shoulder include the development of torsion in the shaft of the humerus to allow an increased range of movement [276, 598], a more distal location of the greater and lesser tuberosities, an increasingly oblique orientation of the scapula with a more laterally directed glenoid, a longer clavicle and a relatively small, round glenoid fossa articulating with a considerably larger surface area on the head of the humerus [107, 134, 276, 598].

A true rotator cuff is distinguished by the blending of individual flat tendons to form a common insertion [598]. Most collagen fibres run longitudinally in this setting, with orthogonally aligned transverse fibres serve to hold the tendons together [598]. Contractions of individual muscles can then exert a pull through their own and also through neighbouring tendons [598]. This arrangement facilitates force transmission across the rotator cuff, allowing more effective and efficient function of the shoulder [598]. The rotator cuff contributes to both the mobility and the dynamic stability of the shoulder. Non-specific recruitment of the muscles of the rotator cuff precedes activity of the deltoid and acts to stabilise the glenohumeral joint by compressing the head on the centre of the glenoid [149, 299, 598]. It has been suggested that the rotator cuff acts as a depressor of the humeral head in addition to actively controlling the fulcrum of humeral rotation [580]. Once movement of the shoulder is in progress, however, the activity of the individual muscles of the cuff becomes more

movement-specific [598]. Although the deltoid and supraspinatus muscles are considered to be the prime movers during abduction, the entire rotator cuff has been shown to act synergistically in this action [598]. The complex biomechanics of the rotator cuff have yet to be completely elucidated. However, it seems reasonable to assume that blending of the individual tendons facilitates this synergy. A blended rotator cuff provides for more precise control of shoulder movement by forming a functional unit, thereby promoting more rhythmical glenohumeral movement and allowing subtle control of more complex patterns such as circumduction.

## DISCUSSION

As with evolutionary change in skeletal anatomy, the morphological changes in the soft tissues have increased the functional advantage of the shoulder.

Each animal model has different advantages and disadvantages for studying tendon pathology and glenohumeral instability. While a nonhuman primate shoulder may offer more anatomic, biomechanical, and immunologic similarity to humans than other animals, cost and management issues make use of this model impractical.

Quadrupedal animals have a weight bearing forelimb and no clavicle, a less-developed acromion, and no coracoacromial arch. These differences between quadrupedal animals and humans are remarkable [644].

The microstructure of the human rotator cuff is one of multiple orthogonally aligned layers, reflecting the multidirectional mechanical stresses applied by the contributing tendons [597]. This complex arrangement is peculiar to the rotator cuff, which appears to be unique to those animals which use their arms overhead for at least part of the time and, as such, is found almost solely in advanced primates. Ethical problems associated with primate research have generally prevented the undertaking of systematic experimental study of the pathology of the rotator cuff in such animals [597].

Models are tools that mimic aspects of human disease. It is clear that animal models have contributed considerably to further our understanding of shoulder pathologies, and have provided novel insights and treatment targets. However, final proof of the use of data determined using these models lies in clinics; time will tell whether model data are predictable for certain targets and, as such, clinical data will help shape and define the models.

There is a significant need for an animal model that allows precise control of postoperative mechanical loading of the repaired tendon-bone interface.

Interestingly, rabbit, rat, dog and sheep, which are the most commonly used animals for assessing pathological and repair mechanisms of rotator cuff lack a true rotator cuff, having individual tendons which did not blend before insertion into the humerus. This

has obvious research implications. Animal models utilising more advanced primates, or perhaps even the tree kangaroo, would be the most relevant to man as they possess a true rotator cuff [598]. However, ethical concerns and costs generally preclude the use of these animals for such researches.

Also, often healthy young animals are used to evaluate healing and remodelling process, which may imprecisely replicate conditions in the often aged human patient who may have osteoporotic bone and multiple comorbidities that may influence healing.

## CONCLUSIONS

Although none of the shoulder of the various animals studied can be regarded as a perfect translational model for human shoulder pathologies, each model reported in this review presents different advantages and disadvantages. This is the reason why, every conclusion arising from animal study must be carefully evaluated in terms of immediate application to the clinical practice. On the other hand, there is no doubt that animal models help us to understand the natural history of various diseases and conditions of the shoulder, and provide a means by which the effectiveness of different therapeutic interventions can be assessed. Further *in vivo* and *in vitro* experiments are still required to enhance our understanding of the models of animal shoulders for research applications.

# Chapter 13

## Summary, discussion, future perspectives and conclusions

## SUMMARY

The aims of this thesis were to study some aspects of the pathogenesis of rotator cuff tears, to evaluate the safety and efficacy of PRFM and double row suture anchor repair techniques to improve healing of the rotator cuff tendon, and to highlight limitations for future research and growing points in the field of rotator cuff scores and animal models for rotator cuff tear. These aims were evaluated using different study designs.

### Tissue engineered biological augmentation for tendon healing

Since tendon healing rate is relatively slow compared with other connective tissues, we reviewed new approaches to improve tendon healing.

This was addressed in chapter 2 by examining the available literature on tissue engineered biological augmentation for tendon healing, including growth factors and cytokines, gene therapy and tissue engineering with mesenchymal stem cells.

Growth factors are signalling molecules involved in cell chemotaxis, proliferation, matrix synthesis, and cell differentiation. They also play an important role in regulation of the phases of tendon healing. After their release from platelets, polymorphonuclear leukocytes, and macrophages in the wound site, growth factors bind to cell surface receptors determining intracellular changes to DNA synthesis and expression, which result in induction of neovascularisation and chemotaxis, along with stimulation of fibroblast proliferation and collagen synthesis.

In animal models, growth factors are effective in increasing the cellularity and overall tissue volume at the repair site. These findings usually result in increased failure loads on biomechanical testing. However, these failure loads become less significant when they are normalized to the volume or cross-sectional area of the repaired tissue. This implies that growth factors are able to improve the strength of the repair by promoting the formation of more scar tissue (i.e., the structural properties are improved but the material properties are not improved). Excessive scar tissue at the healing attachment site may predispose patients to impingement post-operatively [303]. The ultimate outcome of the repair depends on both pullout strength and stiffness. Stiffness and creep may be more important parameters. Ideally, biologic therapies are able to induce tissue formation with material properties close to that of normal tissue [240, 241]

Growth factors can be delivered to the site of injury by direct application. This is the most straight forward method, and can be achieved via local injection, or by using impregnated sutures or scaffolds. Using impregnated sutures or scaffolds has the advantage of delivering the growth factor to the specific area of injury. The disadvantage of overflow loss, associated with local injection, can be avoided with this technique. However, local injection is comparatively non-invasive, simple and quick. The main disadvantage of direct

application is that growth factors only remain at the site for a short duration of time. As that tendon healing continues for months to years, this short duration of growth factor presence may not be effective enough. Nevertheless, several animal studies have demonstrated beneficial results from local injection of growth factors [585].

Gene therapy delivers genetic material (DNA) to cells using viral or non-viral vectors or direct gene transfer. Growth factors have the potential to enhance native repair responses in tendon and ligamentous lesions. However, methods to apply growth factors to the site of injury for extended period are lacking [223]. The transfer of genes which encode healing factors is a challenging solution to this problem. Growth factors, in addition to direct application, can be delivered to the tendon also by gene therapy, as it carries genes encoding growth factors rather than growth factors directly [268, 479, 664]. The cells incorporate the genetic material, and begin to produce growth factors [157]. In this way, the exposure to growth factors is more prolonged. The vectors most frequently used are adenovirus, adeno-associated virus, cationic liposomes, and haemagglutinating virus of Japan-liposomes complexes [268, 479, 664]. Non-viral vectors are less pathogenic, but also less efficient. Viral vectors, in fact, allow the insertion of genes into cells that have ceased to live. This is important in tendons, as tenocytes not divide actively [585]. Potential complications associated with the use of vectors are loss of transgene expression and scarring and adhesion formation secondary to inflammation. Gene transfer using vectors can be achieved via an *in vivo* or *ex vivo* technique. *In vivo* transfer involves direct application of the gene to the relevant tissue. In the *ex vivo* technique, target cells are first removed from the body, before gene transfer is performed in the laboratory. Once successful transfection is achieved, the cells are transferred back into the body. *In vivo* transfection is less invasive and technically easier, and treatment can be commenced during the acute phase of injury. The disadvantage of *in vivo* transfer is non-specific infection of cells adjacent to the site of injury. Furthermore, the success of gene transfer cannot be confirmed, and, in areas of relative cell paucity, only a few cells may be transfected. The use of highly transgenic vectors and injection into areas with a high concentration of cells will ensure transfection of a large proportion of cells. More time is required for *ex vivo* transfection, but this technique avoids the complication of non-specific transfection, allows successful transfection to be confirmed, and also allows *in vitro* expansion of cells if required [585].

Mesenchymal stem cells (MSC) are capable to differentiate into a variety of specialized mesenchymal tissues including bone, tendon, cartilage, muscle, ligament, fat, and marrow stroma [45, 46]. Tissue engineering can be divided into 2 subtypes: the *in vivo* approach and the *ex vivo, de novo* one [268, 479, 664]. The *in vivo* approach permits the self-regeneration of small tissue lesions. The *ex vivo, de novo* approach is designed to produce functional tissue that can be implanted in the body [585]. Tissue engineering is a multidisciplinary field founded on three fundamental principles: the use of healthy multipotent cells that are nonimmunogenic, easy to isolate, and highly responsive to distinct environmental cues;

(2) the development of carrier scaffolds that provide short-term mechanical stability of the transplant and a template for spatial growth of the regenerate tissue; and (3) the delivery of growth factors that drive the process of cell differentiation and maturation [268, 479, 664].

The emerging field of tissue engineering holds the promise to use new techniques for tendon augmentation and repair. Preliminary studies support the idea that these techniques can provide an alternative for tendon augmentation with great therapeutic potential. These techniques are currently at an early stage of development. Whilst these emerging technologies may develop into substantial clinical treatment options, their full impact needs to be critically evaluated in a scientific fashion.

**Tendon augmentation grafts**

Since grafts and biomaterials have been advocated as an effective management option in patients with large and massive rotator cuff tears, we reviewed the current state of knowledge in the field of biomaterials for augmentation of tendon injuries.

This was addressed in chapter 3 by examining the available literature on biomaterials for augmentation of tendon injuries.

In the last few decades, biomaterials have become critical components in the development of effective new medical therapies for wound care [40, 133]. Many new tissue engineered materials have been introduced: artificial polymers, biodegradable films and biomaterials derived from animals or human, using a combination of principles of engineering and biology [40]. As limitations of previous generations of biologically derived materials are overcome, many new and impressive applications for biomaterials are being examined.

Biological scaffolds are protein-based extracellular matrices which usually derive from human or animal connective tissues [114]. Advantages of biological scaffolds are a well-defined 3D surface proteins microstructure (allowing host cell integration), and natural porosity (which provide much larger space for host cell attachment, proliferation, migration and assists gas and metabolite diffusion). These proprieties allow biological scaffolds to quickly interact with host tissue and induce new tissue formation faster than synthetic scaffolds. Limitations of biological scaffolds are low mechanical properties (often resulting in failure of surgery), nonspecific induction ability, undefined degradation rate, variation in biocompatibility depending on the source of raw materials, which can cause inflammatory response and even implant rejection [114].

On the other hand, synthetic scaffolds are manufactured from chemical compounds [114], which permit better control of the chemical and physical properties leading to stronger mechanical strength and consistency in quality. However, biocompatibility of synthetic scaffolds is very poor, as they can never be absorbed or integrated into host tissue. High incidences of postoperative infection, and chronic immune response have been reported with the use of such materials [114].

The ideal scaffold should induce host-tissue ingrowth and tendon regeneration during the process of degradation, which varies dramatically among the commercially available scaffolds [647]. The capability of inducing host-tissue ingrowth is superior when using biological scaffolds, even though this process appears uncontrolled and non-specific [239]. The interaction between scaffold surface and host cells is a key aspect of the use of scaffolds for tendon reconstruction. In the first phase of cellular ingrowth, multiple attachment points are established by the cells through the interaction between transmembrane proteins and proteins at the scaffold surface [114], later strengthened by accumulating integrin receptors, eventually forming a focal adhesion which acts as a connection between the actin cytoskeleton of the cell and the surface [114]. The cell proliferation cycle and cell migration start after the formation of focal adhesions and spreading of cells on the surface [114]. Cell attachment, proliferation and migration is facilitated by the porosity of scaffolds [272]. The surfaces of synthetic scaffold are composed of macromolecules lacking a well-defined structure that allows host cell to produce a strong binding point and start growing.

There is no risk of disease transmission using synthetic scaffolds. A reason of concern is that available scaffolds are produced to mimic the tendon extracellular microenvironment to stimulate cell proliferation and tissue ingrowth, largely ignoring the healing process at the enthesis. The repair procedure often involves reconstruction of the junction, and failure of surgery could be caused by osteolysis and scaffold pullout. Further investigations are required to better understand how to promote the healing of bone–tendon junction. Even though synthetic scaffolds are becoming more popular, well-conducted clinical studies in humans are lacking, and little data describing the complications or adverse events associated with the use of these products are available. Tissue engineering application to synthetic scaffolds may increase their mechanical properties, such as synthetic scaffolds seeded with bone marrow stem cells or tenocytes. However, clinical evidence in this field is scanty.

The emerging field of tissue engineering holds the promise to use biomaterials for tendon augmentation. Preliminary studies support the idea that these biomaterials have the ability to provide an alternative for tendon augmentation. However, available data are lacking to allow definitive conclusion on the use of biomaterials for rotator cuff tendon augmentation. Additionally, the prevalence of postoperative complications encountered with their use varies within the different studies. Rather than providing strong evidence for or against the use of these materials for tendon augmentation, this study instead generates potential areas for additional prospective investigation.

## Novel approaches for the management of tendinopathy

There are several therapeutic options for the management of tendinopathy. However, the best modalities have not been clarified. Therefore in chapter 4 we reviewed the best available evidence for the management of tendinopathy.

Despite an abundance of therapeutic options for tendinopathy, very few randomised prospective, placebo controlled trials exist to assist in choosing the best evidence-based management.

The described success rate of non-operative management of rotator cuff tendinopathies and tears varies widely, from 33% to 92% [75, 77, 84]. Satisfactory results were described in 75% of 53 patients undergoing non-operative treatment at an average follow-up longer than 7 years, particularly those with less than 6 months of pain [77]. In only 62% of 60 patients with documented rotator cuff tears at a minimum of 2 years follow-up was reported as satisfactory, with only 4% considered excellent [676]. In other settings, 70% of 136 patients at an average 1.5-year follow-up had excellent or good results [58].

Even though current evidences are not sufficient to reach clear indications for conservative management of rotator cuff tears, several authors recommend non-operative management for patients with pain without dramatic or progressive weakness [177]. On the other hand, patients with weakness, especially with sudden onset of weakness after an injury, should be investigated for an acute full-thickness tear. In this case, they would benefit of surgery [302]. Earlier surgery has been associated with better outcome, particularly in massive tears [177]. Prognostic factors are clinical presentation, symptoms duration and tear size [58]. These aspects are correlated with each other. Symptom duration less than a year and tear less than 1 cm are predictors of good results with conservative treatment. Rotator cuff repair could be unsuccessful when the tendon retracts beyond the glenoid rim [217, 219, 248]. Moreover, surgery could be contraindicated in elderly population affected by co-morbidities. The elderly are the majority of patients with rotator cuff dysfunction, with marked disabilities leading to functional decline [345].

The conservative approach consists of several interventions commonly applied in clinical practice, but the best programme for conservative treatment is not defined by current evidence.

Physical therapy mainly consists of stretching and strengthening exercise that patients perform at home following a scheduled programme or under the supervision of a physiotherapist [437]. First of all, patient should be educated to modify activities to eliminate the offending motions, such as reaching overhead. An exercise programme should be tailored to the location of the tear, and aim to decrease stiffness and improve function. Such programme would permit to maintain range of motion, prevent adhesions and decrease impingement because of posterior capsular tightness. It is important strengthening the rotator cuff, scapular stabilizers (serratus anterior, rhomboids, latissimus dorsi, and

trapezius), and the deltoid. This would prevent superior humeral head migration during abduction and scapular instability predisposing to impingement syndrome. Progressively, patients will return to their daily activities.

A review of literature did not find any randomised clinical trials about exercise effectiveness in the treatment of full thickness tears of the rotator cuff [7]. Observational studies suggest beneficial effects of physical therapy included in a treatment programme for patient with symptomatic shoulders and radiological or arthroscopic evidence of full thickness rotator cuff tears. However, result of case series investigations are not comparable with each other because of difference in study designs, inclusion criteria, interventions, types of exercise, outcome measurements, follow-up times, and home versus clinic-based programmes. The characteristics of exercise programme are not standardized. Exercises can be specific or general in nature, and their duration, intensity and number of repetitions can be variable. Available evidences do not allow to define benefits closely related to physical therapy. Additionally, the physiological explanation of benefits induced by physical therapy is unclear. Hypotheses are pain modulation, co-ordinated movement performed by the other muscles, placebo, and reduced kinesiophobia. Influence of disease variables, such as traumatic or non-traumatic aetiology and tear size, are not known. Large (3–5 cm) and massive (> 5 cm) full thickness rotator cuff tears generally benefit from surgical intervention [252]. Nevertheless, some patients with massive tears reported functional and pain improvement with conservative treatment [514]. Pain seems to be more important for treatment chose [92, 93, 587].

In clinical practice several intra-articular injections are commonly used for treatment of shoulder pain.

Corticosteroid injections are generally recommended in conjunction with physical therapy and oral anti-inflammatory medications. They should permit rapid pain relief allowing patient to perform physical therapy [591]. A mixture of 3 mL of lidocaine (1%), 3 mL of bupivacaine (0.25%), and a depot corticosteroid is recommended when pain limits patient's ability to perform exercises. Corticosteroid injections have been widely used for different causes of shoulder pain. Reported results are variable, and the literature about corticosteroid injections in patients with rotator cuff tear is limited. No significant benefit were found in patients with partial rotator cuff tear with symptoms lasting longer than six months who had failed physical therapy and a trial of NSAIDs [103]. Corticosteroids act on inflammation, thus acute pathology better responds to their administration. An important concern is the accuracy of delivery the medication into the desired target. Difference in the effectiveness of the intervention performed under radiographic control has been reported in some studies [9, 116, 257]. Others authors found blind injection performed by an experienced orthopaedic surgeon as accurate as ultrasound-guided injection [549]. The effects of corticosteroids effects on the tendon structures limit their use. They can cause collagen necrosis, weakness, and increased risk of rupture [9]. For this reason more than two injections a year, once every 6 months, are discouraged.

Patients with long-term pain may benefit from sodium hyaluronate injection. Evidences about the use of sodium hyaluronate injections in patients with shoulder pain are increasing [278, 331, 547, 588]. Sodium hyaluronate is a normal component of synovial fluid, and contributes to preserve physiological joint friction because of its viscosity [663]. Its efficacy has been proved in small series of patients with rotator cuff tears, with significant benefits over placebo, decreasing pain, the use of oral analgesics, and improves range of motion [588]. As it has no side effects, it seems to be a valid treatment option. However, further studies are needed to confirm its efficacy.

Systemic drugs for patients with shoulder pain from rotator cuff pathology include non-steroidal anti-inflammatory drugs (NSAIDs) [27]. Conventional NSAIDs are commonly prescribed as first line medication. Various types of NSAIDs can be used with similar results [648]. Recently cyclo-oxygenase-2 (COX-2) selective inhibitors have been introduced for the management of shoulder pain. Both NSAIDs and COX-2 selective inhibitors showed short term efficacy in some controlled clinical trials [256, 512, 672]. However both categories of drugs have side effects. Conventional NSAIDs cause gastrointestinal adverse reactions in 8 to 76% of patients [648]. Co-administration of proton pump inhibitor decrease gastrointestinal risk, allowing ulcers to heal and decreasing the risk of ulcer recurrence [188, 258]. NSAIDs also have renal, haematological, dermatological, and neurological side effects [511]. There are some concerns about cardiovascular risk of NSAIDs and COX-2 inhibitors [8, 79, 236, 523]. Long-term therapy, lasting more than two or three months, was associated with increased cardiovascular effects. Selective COX-2 inhibitor showed an increase of vascular events and myocardial infarction compared to naproxen [300]. However, Celecoxib, in doses around 200 mg per day, was not associated with increased cardiovascular risk [426]. Patients showed less epigastric pain than those treated with naproxen [65]. NSAIDs are recommended for short periods. They have several adverse effects and pharmacologic interactions. They interfere with diuretics, β-blockers, angiotensin converting enzyme (ACE) inhibitors, and angiotensin type-2 receptor antagonists, influencing control of blood pressure in patients with hypertension. They can also influence bleeding of patient taking digoxin, anticoagulants and platelet inhibitors [561, 614].

Acetaminophen (paracetamol) can be an alternative for pain relief, with up to 4 g a day associated with a high safety profile. Patients with moderate to severe pain can be treated with a combinations of NSAID or acetaminophen and a fixed dose of opioids [9]. Their safety profile varies from one to another, but they are generally contraindicated in patients with significant respiratory depression and can have significant liability for abuse.

Possible risks and benefits of medications should be taken into account, especially for those patients who are vulnerable. As many patient with shoulder pain are elderly this implications are relevant.

**Histopathology of the supraspinatus tendon in rotator cuff tears.**

Since systemic histopathological studies examining pathological findings and their distribution in rotator cuff tendons are lacking in literature, we evaluated the histopathological features of the macroscopic intact portion of the rotator cuff tendon in patients with a rotator cuff tear.

This was addressed in chapter 5 by examining the histopathological features of the macroscopic intact portion of surgical specimens of supraspinatus tendon from patients with rotator cuff tears.

Tendon samples were harvested from 88 individuals who underwent arthroscopic repair of a rotator cuff tear, and from 5 male patients who died of cardiovascular events. A full thickness supraspinatus tendon biopsy was harvested en bloc within the arthroscopically intact middle portion of the tendon.

The mean pathologic sum-score of ruptured tendons was significantly greater than the mean pathologic score of control tendons. Within each specific category of tendon abnormalities, the control and ruptured tendons were significantly different.

**Light microscopic histology of supraspinatus tendon ruptures**

Since there is no consensus on the distribution of histopathological changes in rotator cuff tears, we compared the histopathological features of the gleno-humeral and subacromial portions of the rotator cuff.

This was addressed in chapter 6 by examining the histopathological features of surgical specimens of supraspinatus tendon from patients with rotator cuff tears.

Tendon samples were harvested from 31 subjects who underwent arthroscopic repair of a rotator cuff tear, and from 5 male patients who died of cardiovascular events. Within each specific category of tendon abnormalities, the chi square test showed significant differences between the control and ruptured tendons. We found thinning and disorientation of collagen fibers and chondroid metaplasia to be more pronounced on the articular side of the specimens from patients with rotator cuff tear.

**Higher fasting plasma glucose levels within the normoglycemic range and rotator cuff tears**

Since there is a possible relationship between hyperglycaemia and collagen structure alterations, we evaluated the role of higher fasting plasma glucose levels within the normoglycemic range in developing a rotator cuff tear.

This was addressed in chapter 7 by performing a frequency-matched case-control study of the plasma glucose level obtained from non-diabetic patients undergoing arthroscopic

rotator cuff repair, and compared with a matched control group of patients of a similar age.

The study included 194 subjects who were operated at our institution. Group 1 included 97 consecutive patients who underwent arthroscopic repair of a rotator cuff tear. Group 2 (control group) included 97 patients who underwent arthroscopic meniscectomy for a meniscal tear in the same period, and had no evidence of shoulder pathology. These patients were frequency-matched by age (within 3 years) and gender with patients of Group 1.

Patients with rotator cuff tears showed statistically significantly higher fasting plasma glucose levels within the normoglycemic range when compared to patients with meniscal tear.

**Triglycerides and total serum cholesterol in rotator cuff tears: do they matter?**

Since a relationship between high serum lipid concentration and complete rupture of the Achilles tendon has been suggested, we evaluated the role of serum triglyceride concentration and total serum cholesterol concentration in developing a rotator cuff tear.

This was addressed in chapter 8 by performing a cross-sectional study of the serum triglyceride concentration and total serum cholesterol concentration in patients undergoing arthroscopic rotator cuff repair, and compared them with a control group of patients of a similar age.

The study included 240 subjects. Group 1 included 120 patients who underwent arthroscopic repair of a rotator cuff tear. Group 2 (control group) included 120 patients who underwent arthroscopic meniscectomy for a meniscal tear, and had no evidence of shoulder pathology. These patients were frequency-matched by age (within 3 years) and gender with patients of Group 1.

When comparing the two groups, there was no difference either in serum triglyceride concentration or total serum cholesterol concentration.

**Equivalent Clinical Results of Arthroscopic Single-Row and Double-Row Suture Anchor Repair for Rotator Cuff Tears: A Randomized Controlled Trial**

Since restoring the anatomic footprint of the rotator cuff has been proposed to improve the healing and mechanical strength of repaired tendons, we compared single and double row suture anchor techniques [198].

This was addressed in chapter 9 by performing a randomised controlled trial to compare the clinical and structural outcome of single versus double row suture anchor repair of a rotator cuff tear [198].

We recruited 60 patients. In 30 patients, rotator cuff repair was performed with single row suture anchor technique (Group 1). In the other 30 patients, rotator cuff repair was

performed with double row suture anchor technique (Group 2). 8 patients (4 in the single row anchor repair group and 4 in the double row anchor repair group) were lost at follow up.

At the 2 year follow-up, no statistically significant differences were seen with respect to the UCLA score and ROM values.

**Platelet-rich plasma augmentation for arthroscopic rotator cuff repair: a randomised controlled trial**

Since platelet-rich plasma (PRP) and platelet-rich fibrin matrix (PRFM) have been proposed to improve rotator cuff tendon healing, we evaluated the efficacy and safety of PRFM for augmentation of RC repair.

This was addressed in chapter 10 by performing a randomised controlled trial to compare the clinical and structural outcome of PRFM augmented versus non augmented suture anchor repair of a rotator cuff tear.

Eighty-eight patients with a rotator cuff tear were randomly assigned by a computer-generated sequence to receive arthroscopic rotator cuff repair without (n = 45) or with (n = 43) augmentation with autologous PRFM. There was no statistically significant difference in total Constant Score when comparing the results of arthroscopic repair of the 2 groups. There was no statistically significant difference in MRI tendon score when comparing arthroscopic repair with or without PFRM.

**Instruments to assess patients with rotator cuff pathology: a systematic review of measurement properties**

Since several shoulder scores are available to evaluate patients with shoulder pain, we evaluated the methodological quality of studies on the measurement properties of rotator cuff questionnaires.

This was addressed in chapter 11 by performing a COSMIN systematic review of the literature on the measurement properties of rotator cuff questionnaires to describe how well various aspects of the design and statistical analyses of studies on measurement properties are performed [374].

A systematic review of published studies on the measurement properties of rotator cuff questionnaires was performed. Two reviewers independently rated the quality of the studies using the Consensus-based Standards for the selection of health Measurement Instruments (COSMIN) checklist. This checklist was developed in an international Delphi consensus study. 16 studies were included, in which 2 measurement instruments were evaluated, namely the Western Ontario Rotator Cuff Index (WORC) and the Rotator Cuff Quality-of-Life Measure (RC-QOL). The methodological quality of the included studies was

adequate on some properties (construct validity, reliability, responsiveness, internal consistency, and translation) but need to be improved on other aspects. The most important methodological aspects that need to be developed are as follows: measurement error, content validity, structural validity, cross-cultural validity, criterion validity, interpretability.

### Animal models for translational research on shoulder pathologies: from bench to bedside

Since the absence of validated animal models for the study of shoulder pathology challenges the research on this field, we evaluated the role of different animal models in rotator cuff research.

This was addressed in chapter 12 by performing a review to evaluate animal models of rotator cuff tear.

*In vitro* models, consisting of cadaveric specimens, are useful in providing basic understanding of the functioning of the shoulder and for biomechanical experiments. *In vivo* models provide the means to model living phenomena, such as tendon healing process, tendinopathy, instability and adaptive responses to surgery. However, intrinsic differences among different species make translation to human shoulder pathologies difficult. Most of the animals used in experimental settings are quadrupeds, using the forelimbs for weight-bearing during locomotion, with no or minimal overhead activity. The various animal models already used to study shoulder pathologies are presented in this article. However, there is a lack of validation for these animal models, which provides challenge to the further research in this field.

## DISCUSSION AND FUTURE PERSPECTIVES

### Pathogenesis of rotator cuff tears

Rotator cuff tears are common and lead to shoulder pain and functional impairment. Despite their frequency and related disability, the aetiology and pathogenesis are still debated. Multiple factors contribute to tears of the rotator cuff tendons. Classically, the underlining mechanisms are subdivided into extrinsic and intrinsic. Extrinsic factors are anatomic variables, such as acromial morphologic characteristics, os acromiale and acromial spurs which compress tendons by bony impingement or direct pressure from the surrounding soft tissue [20, 423], and environmental factors, such as shoulder overuse, smoking, and any medical condition that impairs the inflammatory and healing response such as diabetes mellitus [463]. Intrinsic factors arise from the tendon itself, because of tensile overload, aging, or microvascular supply, traumatisms, or degeneration [20]. It is controver-

sial whether any of these mechanism is primary or secondary, and in many patients there seems to be an interaction between them [20].

New risk factors, such as glucose and lipids, have been investigated. However an univocal conclusion has not been reached, and further studies are needed to understand their possible role, range of risk and potential prevention.

**Extrinsic factors**

Extrinsic factors have been proposed to be a cause for rotator cuff disease because of tendon compression through bony impingement and surrounding soft tissue. Acromial morphology, presence of spurs, morphology of the coracohumeral ligament and presence of osteophytes at the acromioclavicular joint are the main anatomical elements that have been investigated.

The first observations arose from the intraoperative finding of acromial impingement [458]. Neer et al [458, 459] noticed that tendinopathy and rupture mainly occur in the supraspinatus tendon, in an area that abutted against the coraco-acromial ligament, the anterior acromion and sometimes the acromio-clavicular joint during forward elevation. They concluded that 95% of all the rotator cuff tears were initiated by impingement associated lesions.

Successively, acromial shape was classified in tree types and correlated with rotator cuff tears [68]. Hooked, curved, and laterally sloping acromions are associated with cuff tears. On the other hand, flat acromions were not associated with rotator cuff tendon tears [68, 386]. Acromial shapes can be both congenital and acquired. Age determines a progression from a flat to a curved or hooked acromion [665], possibly because of traction forces [578]. This would partially explain the epidemiological evidence of higher incidence of cuff tears with increased age, but also suggests a primary intrinsic initiating factor. An already damaged rotator cuff could induce the progression to a hooked acromion because of increased stress on the coracoacromial arch. Most partial thickness tears are intrasubstance or on the articular side, and not on the upper bursal side, where mechanical abrasion from the acromion should act [355, 468, 555]. Moreover, other studies reveal that the majority of rotator cuff tears are degenerative in nature, preceding acromial degeneration [483]. It has been suggest that superior migration of the humeral head, following failure of the rotator cuff, could be responsible for acromial degeneration [468, 637, 681]. From the observation of the preponderance of joint side rotator cuff pathology, a new model of impingement (internal impingement, or superior and posterosuperior impingement) has been proposed, but evidence are still incomplete. The superior aspect of the glenoid fossa and the greater tuberosity of the humerus would be responsible for rotator cuff compression [169].

Acromial bony spurs have been proposed to be associated with rotator cuff tears, but their etiology and role in the disease has been long debated [57]. Spurs are located at the insertion of the coracoacromial ligament on the acromion. They are an enthesopathy,

probably from strain in the coracoacromial ligament. Examination of the undersurface of the acromion lead to conclude that acromial spurs are a secondary phenomenon induced by primary bursal-side cuff tear [106, 483]. The increased volume of subacromial tissues would increase coracoacromial ligament strain and lead to acromial changes [558].

**Intrinsic factors**

Intrinsic factors act within the tendon itself. Tensile overload, aging, microtrauma and microvascular supply are implied in the pathogenesis of rotator cuff disease. Given the higher number of tears on the articular side, histological and biomechanical studies analyzed tensile load of joint and bursal side fibres, showing a smaller cross sectional area and a greater vulnerability of articular side fibers, especially when the shoulder is in the elevated position [348, 454]. The heterogeneity of tendon and the difference in its mechanical stress shear, may contribute to tendon failure [66].

The role of ageing in the development of rotator cuff disease is supported by epidemiological studies. The frequency of tears increases from younger to older population, affecting 31% of patients over 70 years [94]. However, there is little proof that tendons from healthy, older persons exhibit histological evidence of degeneration. Unruptured rotator cuff tendons, even at an advanced age, and ruptured supraspinatus tendons are clearly part of 2 distinct populations. Histological age-related characteristic associated with rotator cuff tears include: thinning and disorientation of the collagen fibers, myxoid degeneration, hyaline degeneration, chondroid metaplasia, calcification vascular proliferation, fatty infiltration [251]. The last two reflect reparative process streaming from the bursal side, where they are more common to be seen. The others represent degenerative changes that reduced tensile capacity [296].

The degenerative-microtrauma model is the most reliable: age-related tendon damage compounded by chronic microtrauma results in partial tendon tears that then develop into full rotator cuff tears [463].

Microtrauma theory suggests that insufficient time is given between repetitive stresses to allow healing of small injuries that worse. Moreover, remaining fibers undergo an increased load resulting in higher risk of failure [418].

Microvascular supply is another factor that has been debated. The so called “critical” zone is an hypovascular area proximal to the insertion of the supraspinatus tendon [356]. However, it is controversial whether the “critical zone” really contributes to tendon pathology, because histological and immunohistochemical analyses of torn rotator cuff tendons [203] and intraoperative laser Doppler flowmetry [619] showed relative hyperperfusion at the area of the critical zone and at the tear edge. Nevertheless, when the arm is in full adduction, and the supraspinatus is compressed by the humeral head, reduced perfusion may be significant.

## Genetics

Investigations into the genetic factors involved in the aetiology of tendinopathy are still in their infancy [409]. Recently, studies have suggested the contribution of genetic factors in the pathogenesis of rotator cuff tears. Results from a prospective, cross-sectional study, based on clinical information of patients with full-thickness tears, their siblings and matched controls have shown that, in siblings, the risk of developing tears of the rotator cuff is more than twice, with five times the chance to experience symptoms [249]. Thus, there is a significant genetic susceptibility towards the development of full-thickness tears. Its phenotypic expression arises at the ultrastructure of the tendon, and may operate through apoptosis and regenerative capacity. Genetics may predispose to tendinopathy, traditionally thought to be an age-related process. Patients stratified by family history but matched for age, gender and environmental conditions have shown significant differences in their prevalence of cuff tears. Therefore, it is possible that only those individuals with a genetic predisposition may evolve age-related degeneration. Genetic susceptibility seems to influence the presentation of symptoms. Any point of the sensorineural pathway of the cuff can represent the genetic basis for pain.

The same genetic factors that predispose to the development of rotator cuff teas in siblings of patients, influence their progression [243]. Tear size is more likely to progress in sibling than in control population over a period of five years. There is a greater risk for a tear to be painful if it presents in a sibling of a patient with a painful tear. This supports the heritable component of this ailment [98, 194].

Despite these preliminary evidences of genetic component in tears of the rotator cuff, specific genes have not been identified [577]. This results from the fact that rotator cuff tear is a complex multifactorial conditions determined by interaction of multiple gene products and environment. Structural genes, such as tenascin C (TNC) and collagen V a 1 (COL5A1), have been already associated with Achilles tendinopathy and Achilles tendon ruptures, and are candidate to be investigate in rotator cuff. In the future, tendon injury could be prevented by the identification of predisposing genes.

## Rotator cuff tendon healing

The attachment of tendon to bone presents a great challenge in tissue engineering, because a soft compliant material (tendon) attaches to a stiff (bone) material. A high level of stress is expected to accumulate at the interface due to the difference in stiffness of the two materials. This problem is solved by the presence of a unique transitional tissue called “enthesis” at the interface which can effectively transfer the stress from tendon to bone and vice versa through its gradual change in structure, composition and mechanical behavior.

Tendon healing to bone can be theoretically enhanced by 2 approaches: biomechanics and biology.

§ *Biomechanics*

Rotator cuff surgery aims to provide tendon fixation secure enough to hold the repaired tendon in place until biological healing occurs [384]. Healing of repaired rotator cuff tendons will be helped by appropriate restoration of the anatomic footprint and constructs providing adequate compression of the tendon on the footprint itself [32].

With advances in arthroscopic surgery, several techniques have been developed to increase the tendon-bone contact area, reconstituting a more anatomic configuration of the rotator cuff footprint and providing a better environment for tendon healing.

Earlier repair methods of single-row suture anchorage do not completely recreate the native footprint insertion of the supraspinatus tendon onto the greater tuberosity, leading to incomplete anatomic healing.

As we re-attach tendinous tissue to bone, theoretically only the re-constitution of enthesial fibrocartilage would guarantee an optimal outcome. To improve rotator cuff tendon healing, rotator cuff fixation strength has been extensively studied. To optimize the healing process, it seems to be important to attempt restoration of the original anatomy of the insertion of the rotator cuff, which would provide larger area for bony incorporation and healing, and to develop constructs that provide increased compression of the tendon on the footprint which may affect the mechanical strength and function of the repaired tendon. This is especially important at the early stages of rehabilitation, when the tendon-bone interface is still weak, and complete functional recovery has yet to take place. With the development of new biological enhancement techniques, it might prove important to maintain a large area of contact between tendon and bone, allowing more fibers to participate in the healing process. This could be theoretically obtained by a double row repair.

Our randomised controlled trial [198] shows that there are no advantages in using a double row suture anchor technique to restore the anatomical footprint. The mechanical advantages evidenced in cadaveric studies do not translate into superior clinical performance when compared with the more traditionally, technically less demanding, and economically more advantageous technique of single row suture anchor repair.

Reconstructions of the tendon-to-bone unit for full-thickness tears in either single- or double-row technique differ with respect to several endpoints.

Double-row repairs are associated with increased consumption of material and surgery time as well as with a demanding learning curve regarding technical skills of the surgeon. On the other hand, new techniques may help to achieve a high initial fixation strength with only a small extension of operating time.

Double-row techniques allow widely anatomic reconstruction of the tendon insertional area as a precondition for theoretically solid ingrowth. Increased footprint coverage and restoration may therefore facilitate the biologic regeneration and healing. However, as an essential limitation of the available biomechanical studies is that repair techniques were tested in (animal) cadaver models which may not reflect the findings in daily practice of rotator cuff surgery since tendon tissue often shows degeneration and fatty infiltration. Furthermore, most studies were performed at time zero and cannot predict the long-term stability of any construct as increased contact pressure to the tendon-to-bone-unit may account for tissue malnutrition and may deteriorate the healing process.

The assumption of tissue strangulation through strong tissue fixation using double-row techniques is possible. On the other hand, no increased complication rates were observed with double- row technique. Available radiographic follow-up studies suggest a beneficial effect of double-row reconstruction on structural integrity of the reattached tendon or reduced recurrent defect rates, respectively [497].

Newer arthroscopic suture bridge techniques of rotator cuff repair have been proposed to develop constructs which provide increased compression of the tendon on the footprint [96, 353, 384, 428, 492-495].

The suture tension for the transosseous technique provides a more direct tendon-to-bone compression vector. In contrast, the sutures for the suture anchor technique predominantly provide circumferential tension around the tendon but relatively little compression between tendon and bone [494, 495]. Suture bridges provide significantly more compression compared with suture anchor techniques. Improved pressure characteristics with a transosseous technique may allow for improved tendon-to-bone healing and a lower persistent tear rate.

The transosseous-equivalent rotator cuff repair technique has been developed to optimize healing biology at a repaired rotator cuff tendon insertion [493-495]. This technique for arthroscopic repair of rotator cuff tears improves the contact area and the mean footprint pressures, without compromising the bony footprint by the distal-lateral fixation. It also produces a low-profile repair, sharing the load with the suture-bridge technique between fixation points, which maximizes the strength of the repair [32]. The repair involves inserting a medial row with suture anchors that utilize mattress repairs.

Many studies have shown comparable or superior initial fixation strength for suture anchor repairs. Apreleva et al [32] and Park et al [492] showed that an improved repair site area is obtained with a transosseous repair technique when compared to suture anchor techniques.

The ‘‘transosseous equivalent’’ or ‘‘SutureBridge’’ technique utilizes a medial row of suture anchors with a single mattress configuration, respectively. We described the Roman Bridge, an arthroscopic rotator cuff repair technique which uses suture bridges to optimize rotator cuff tendon–footprint contact area and mean pressure. The Roman Bridge technique can also be associated to a soft tissue tenodesis.

The original Roman bridge technique was a high profile repair [196]. Successively, we described the low profile Roman bridge technique (single pulley – suture bridges) for knotless double row repair of the rotator cuff, a modification of our previous technique [368]. The Roman Bridge (double pulley - suture bridges) technique maximizes the advantages of the two techniques. The double pulley technique provides an extremely secure fixation in the medial aspect of the footprint [96, 354, 440]. The suture bridges allow to improve pressurized contact area and mean footprint pressure, to not compromise the bony footprint by the distal-lateral fixation, produce a low-profile repair, share the load between fixation points, which maximizes the strength of the repair and to provide a barrier of synovial fluid from the healing zone involving tendon and bone [493-495].

Our technical skills evolve, together with a better understanding of the biomechanics and biology of tendon to bone healing. In the future, comparison of new double row suture bridge techniques versus single row and old double row techniques is required [392].

*§ Biology*

Several challenges exist in developing an effective biologic therapy to augment rotator cuff healing. First, the most effective growth factor or combination of growth factors must be determined. As research progresses, it is clear that a single factor therapy may not be sufficient. Rather, it is probable that several factors may be necessary, and the various possible combinations are numerous.

The second challenge is determining the optimum time for growth factor delivery. Growth factors are upregulated during the healing process in a temporal fashion, with most growth factors being upregulated 1 week following repair in rat models. In the first week after injury/repair, the healing process is in the inflammatory phase. It is possible that this inflammatory response may override any anabolic agent that is added at this time. Therefore, timing of growth factor application is critical.

Addition of PDGF into a rat patellar tendon defect at 3 days had no effect on the biomechanical strength of the repair, whereas PDGF injection on day 7 improved peak loads-to-failure. Therefore, any growth factor added at the time of surgery needs to be incorporated into a sustained-release drug delivery vehicle that ensures that the factor is present during the regenerative phase of healing.

The final challenge involves developing a delivery vehicle for the growth factor. Many rotator cuff repair surgeries are now performed arthroscopically, so the delivery vehicle must be amenable to placement through cannulas and the growth factor must not be eluted in the fluid-filled arthroscopic environment. These technical considerations make gels, pastes, cements, and glues less desirable than scaffolds or patches.

Our randomised controlled trial does not support the use of autologous platelet-derived growth factors in the form of PRFM for augmentation of a double row repair of a small or medium RC tear to improve the healing of the RC. We did not demonstrate superior clini-

cal or structural performance when compared with the more traditionally, technically less demanding, and economically more advantageous technique of non augmented suture anchor repair. Our results are applicable to small and medium RC tears: it is possible that the use of autologous growth factors contained in platelet rich plasma may be beneficial for large and massive RC tears. Also, given the heterogeneity of platelet rich plasma preparation products available on the market, it is possible that other preparations may be more effective.

PRP has been recently used in other clinical studies to enhance the healing process after repair procedure of rotator cuff tears. To date, only other two randomized controlled trials have been performed. Randelli et al [520] randomized fifty-three patients with a full-thickness rotator cuff tear to receive arthroscopic rotator cuff repair without (n = 27) or with (n = 26) injection of activated PRP combined with autologous thrombin between the bone and the repaired rotator cuff. The arthroscopic repair was performed by using a single-row technique with bioabsorbable suture anchors (Bio-Corkscrew; Arthrex, Naples, FL, USA). Authors demonstrated that the PRP provide an early pain relief, starting from day 3 after surgery, and a shorter functional recovery when compared to a control group. There was a statistically significant difference between two groups for all clinical outcomes at the 3-month follow-up, including the Constant score, the University of California at Los Angeles (UCLA), the Simple Shoulder Test (SST) and the strength in external rotation (SER). Moreover, they found any statistically significant difference in terms of tendon re-rupture rates, although the control group reported higher rates (52% vs 40%).

Rodeo et al [538] (http://clinicaltrials.gov/ct2/show/NCT00198185?term=cascade&rank=4) randomized sixty-seven patients with a full-thickness rotator cuff tear to receive arthroscopic rotator cuff repair without (n = 31) or with (n = 36) application of PRFM construct at the bone-tendon interface, with a minimum 1-year follow-up. Authors did not reported any advantages for group managed with platelet-rich fibrin matrix in terms of tendon healing and vascularity, and clinical outcomes, including manual muscle strength and clinical rating scales (ASES and L'Insalata). Moreover, they found higher re-tear rates in the PRFM group (33% vs 29%).

Jo et al [287] performed a prospective cohort study (level II), in which forty-two patients with a small to massive full-thickness rotator cuff tear were randomized to receive arthroscopic rotator cuff repair without (n = 23) or with (n = 19) application of PRP gels. The arthroscopic repair was performed with a suture bridge technique with bioabsorbable suture anchors (Bio-Corkscrew; Arthrex, Naples, FL, USA). Biological approach consisted in applying PRP gels (three per patient) at the repair site between the torn tendon and the bony insertion. Authors did not find any significant differences between two groups in VAS score and functional outcome measures, such as the Constant score, the UCLA, the SST, the American Shoulder and Elbow Surgeon (ASES) score, the Disabilities of the Arm, the Shoulder and Hand (DASH) score, and the Shoulder Pain and Disability Index (SPADI)

score. The tendon re-rupture rate in the control group was found higher than in the PRP group (41.2% vs 26.7%), but there was any statistically significant difference. According to their findings, authors did not recommend the PRP application during arthroscopic rotator cuff repair.

Barber et al[54] performed a case-control study (level III) in which forty patients with a small to large full-thickness rotator cuff tear were randomly assigned to receive arthroscopic rotator cuff repair without (n = 20) or with (n = 20) augmentation with autologous platelet-rich fibrin matrix (PRFM). The arthroscopic repair was performed by using a single-row technique with bioabsorbable suture anchors. The biological augmentation consisted in suturing two autologous PRP constructs (PRFMs) for each patient in the repair site between the tendon and the humeral footprint at the greater tuberosity. Authors reported a statistically significant difference between the two groups only in the Rowe score, but not in the other scores such as the ASES score, the Single Assessment Numeric Evaluation (SANE) score, the SST, and the Constant scores. Moreover, they found a statistically significant difference in tendon re-rupture rates in the control group compared with the PRP group (60% vs 30%) at MRI assessment. Authors concluded that PRFM augmentation of repair procedure results in lower risk of re-tear without improvement of functional outcomes, except the Rowe score.

Increased knowledge of tendon healing has induced great expectation on PRP therapy as it allows topical release of molecules implied in the biological process. Numerous biochemical mediators of PRP effects has been identified. However molecules involved are much more and need to be better described in their implication on tendon healing. Moreover, the complexity of the healing process needs to be taken into account. Its further comprehension would be helpful to fully understand PRP potentiality and to optimize its composition and therapeutic applications. Available PRP products differ in platelets, leucocytes and fibrin network. Those elements influence PRP biological characteristics and need to be taken into account comparing studies results. Animal studies have confirmed biological expectancy of PRP. However preclinical studies on rotator cuff models still need to be performed.

The effectiveness of PRP to obtain the pain relief is controversial. Randelli et al[520] reported a significantly earlier decrease of VAS score values in the PRP group compared with the control group. On the other hand, Jo et al[287] did not find any significant difference between the two groups for any value of VAS score at any time point of follow-up.

No studies showed significant difference in postoperative tendon healing at MRI or ultrasound imaging. Other than Barber et al[54], all authors reported no statistically significant difference in tendon re-rupture rates between the control group compared with the PRP group.

Although experimental evidences support the effectiveness of PRP and growth factors to enhance the tendon healing process [383, 539], these findings do not recommend the PRP application during the rotator cuff repair.

## CONCLUSIONS

The pathogenesis of rotator cuff tears is still debated. Understanding the mechanism of rotator cuff pathology would facilitate the rationale for therapeutic interventions, by guiding the design, selection and implementation of treatment strategies, such as biologic modulation and preventive measures. The results from this thesis clearly outline that rotator cuff tears are not just a localised problem, but they are a "systemic disease" of the tendon. These findings have implication both in research settings and patient clinical outcomes.

None of the shoulder of the various animals commonly used in research setting can be regarded as a perfect translational model for human rotator cuff pathologies. Each model presents different advantages and disadvantages. On the other hand, there is no doubt that animal models help us to understand the natural history of various diseases and conditions of the shoulder, and provide a means by which the effectiveness of different therapeutic interventions can be assessed. While a nonhuman primate shoulder may offer more anatomic, biomechanical, and immunologic similarity to humans than other animals, cost and management issues make use of this model impractical. Researchers should remember that every conclusion arising from animal study must be carefully evaluated in terms of immediate application to the clinical practice.

Evaluation of outcomes is extremely important when the outcome being measured is subjective, as in the assessment of rotator cuff pathology. In this field, there is room for improvement in the methodological quality of studies on measurement properties. Authors of future studies on questionnaires for rotator cuff should consider the COSMIN checklist to evaluate the methodological quality of study protocol before performing the study.

Although new appealing techniques of double row repair and biological augmentation have been proposed to enhance rotator cuff tendon healing, these approaches did not result in improved clinical or radiological outcome in randomised controlled trials. Therefore, shoulder surgeons should consider results from level I studies, before to change their surgical options for patients with rotator cuff tears. Application to clinical practice of results obtained from well conducted level I randomised controlled trials remains the best way to move from opinion-based to evidence-based orthopaedic practice.

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# Short CV

Umile Giuseppe LONGO was born in Cosenza, Italy, on October 8th 1979. In 1998 he graduated from high school (Gymnsium, Luzzi, Cosenza) and started to study medicine at University Campus Bio-Medico of Rome. He received his medical degree in 2004 with a thesis performed under the supervision of Prof V. Denaro in the laboratory of experimental orthopaedics of Pamplona-Spain (Head Prof F. Forriol). The author completed the residency in orthopaedics at University Campus Bio-Medico of Rome (Head: Prof V. Denaro) in 2009. He performed a second Level Master in "Metabolic bone diseases", awarded by the University of Florence, Italy in 2009 (Head: Prof M.L. Brandi). He performed 2 research sports medicine fellowships in Stoke-on-Trent (Head: Prof N. Maffulli) and a shoulder fellowship in 2010 in London (Heads: Mr A. Wallace and P.M. Ahrens).

The present thesis has resulted in several peer-reviewed publications and 4 international awards.

The author has been the recipient of several prestigious national and international awards: (2006 BASEM (British Association of Sports Exercise Medicine) Research Award; Young Researcher Award for 2008 at ESSKA (European Society of Sports traumatology, Knee surgery, and Arthroscopy) for the paper "Histopathology of the supraspinatus tendon in rotator cuff tears", given to the best scientific manuscript in the fields of Knee Surgery, Sports Traumatology and Arthroscopy presented by a researcher < 40 years of age; 2008 EFAS (European Foot & Ankle Society) Travelling Fellowship; 2008 Hughston Award of the AOSSM (American Orthopaedic Society for Sports Medicine) for the paper entitled "Equivalent Clinical Results of Arthroscopic Single-Row and Double-Row Suture Anchor Repair for Rotator Cuff Tears: A Randomized Controlled Trial", given for the most outstanding paper that appeared in the *American Journal of Sports Medicine* in 2007; 2009 ICRS (International Cartilage Repair Society) Scholarship; 2009 European Arthroscopy Travelling Fellowship of the SIGASCOT-AGA-SFA-AEA; 2009 Europe/Japan travelling fellowship of the European Society for Surgery of the Shoulder and the Elbow (SECEC/ESSSE); 2010 European Federation of National Associations of Orthopaedic Sports Traumatology (EFOST); 2010 Anglo-Dutch travelling fellowship; 2010 Helal and Harries prize - Sport & Exercise Medicine Section of the Royal Society of Medicine for the paper "Platelet-rich plasma augmentation for arthroscopic rotator cuff repair: a randomised controlled trial"; 2011 ESSKA (European Society of Sports traumatology, Knee surgery, and Arthroscopy) - SLARD (Latin American Society of Knee Arthroscopy and Sports Medicine) travelling fellowship; 2011 EFORT foundation visiting fellowship; 2011 SECEC/ESSSE research grant.

Dr Longo is author of about 200 peer reviewed manuscripts, co-author of a monography "*Bursitis*", BMJ Knowledge Point of Care Project, and co-author of about 50 chapters published in international books.

Dr Longo is Associate Editor of BioMed Central Musculoskeletal Disorders since 2009 and Deputy Section Editor for the Biomechanics and Orthopaedics section of BioMed Central Musculoskeletal Disorders since 2010.

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