UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Centre hospitalier
universitaire vaudois

Mémoire de Maîtrise en médecine 3271

Rotator cuff repair *in vivo* with scaffolds, cells and/or growth factors

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Lausanne, 15.12.2015

Preface

In a pilot study, we did some in vitro testing that aimed to evaluate the biomechanical properties of silk scaffolds that could potentially be used for tendon repair; we also analyzed the effects of growing cells within these matrices. The results are reported at the end and were not satisfactory for the biomechanical properties necessary for a tendon matrix substitute. This led us to the idea of writing the following review in order to help research focusing on the most effective strategies to help in development of innovative strategies for tendon healing and repair, in particular rotator cuff tendons.

Acknowledgments

The moment I was informed that I could accomplish my Master's Thesis within the Unit of Regenerative Therapy I was very pleased and motivated. I would like to thank Anthony Grognuz for the counseling and teaching throughout my work, Prof. Lee-Ann Laurent-Applegate for the warm welcome and the readiness to help, and Prof. Alain Farron for kindly accepting to be the expert of this project. A big thank you also to all the Unit of Regenerative Therapy where all the people were very kind and I really had a good experience for this phase of my medical formation.

List of abbreviations

Throughout this document many abbreviations will be used in order to render the text easier to read. Here is a comprehensive list of these abbreviations.

ADM: acellular dermal matrix
ADSCs: adipose-derived stem cells; also known as adipose-derived stromal/stem cells (ASCs)
B: biomechanics
BG: bioactive glass (60% SiO_2, 36% CaO, 4% P_2O_5)
BM-MSCs: bone marrow-derived mesenchymal stem cells
BMP-2: bone morphogenetic protein 2
Ca-P: calcium-phosphate
CPS: $Ca_5(PO_4)_2SiO_4$
d: days
ECM: extracellular matrix
EGR1: early growth response 1 transcription factor
FGF-2: fibroblast growth factor 2; also known as basic fibroblast growth factor (bFGF)
Fill.: filling
G-CSF: granulocyte colony stimulating factor
GDF-5: growth differentiation factor 5; also known as cartilage-derived morphogenetic protein 1 (CDMP-1) or bone morphogenetic protein 14 (BMP-14)
GDF-6: growth differentiation factor 6; also known as cartilage-derived morphogenetic protein 2 (CDMP-2) or bone morphogenetic protein 13 (BMP-13)
GDF-7: growth differentiation factor 7; also known as cartilage-derived morphogenetic protein 3 (CDMP-3) or bone morphogenetic protein 12 (BMP-12)
H: histology
HA: hyaluronan
HBDS: heparin/fibrin-based delivery system
IGF-1: insulin-like growth factor 1
Interpos.: interposition
IS: infraspinatus tendon
MDCs: muscle-derived cells
MT1-MMP: membrane type 1 matrix metalloproteinase; also known as matrix metalloproteinase 14 (MMP-14)
NS: not specified
Overlap.: overlapping
P4HB: poly-4-hydroxybutyrate
PBS: phosphate-buffered saline
PC-PE: polycarbonate-polyurethane
PCL: poly(ε-caprolactone)
PD: porcine dermal collagen
PDGF-BB: platelet-derived growth factor BB
PEGDA: poly(ethylene glycol) diacrylate
PEO: poly(ethylene oxide)
PGA: polyglycolic acid
PLA: polylactic acid
PLAGA: poly(85 lactic acid-co-15 glycolic acid)
PLC: poly-L-lactate-ε-caprolactone
PLG: poly(D,L-lactide-co-glycolide)
PLGA: polylactide-co-glycolide
PLLA: poly(L-lactic acid)
PPCs: periosteal progenitor cells
PRP: platelet-rich plasma
PTFE: polytetrafluoroethylene
PU: polyurethane
SAP: self-assembled peptide
Scx: scleraxis
SIS: small intestine submucosa
SS: supraspinatus tendon
SSc: subscapularis tendon
SVFCs: stromal vascular fraction stem cells
TGF-β: transforming growth factor beta
TGIF1: TGFβ-Induced Factor Homeobox 1 gene
TSG-6: tumor necrosis factor α-induced gene/protein 6
TSPCs: tendon stem/progenitor cells; also known as tendon-derived stem cells
VPG: vesicular phospholipid gel
w: weeks

Abstract

Background: Rotator cuff tears are very common and failure of the repair is still a problem. There are many new techniques that are currently being investigated in animal models in order to improve the outcomes of tendon repair but no systematic review has been accomplished to date.

Objectives: To review the literature concerning the materials, cells and growth factors that are available for rotator cuff repair *in vivo* on animal models. This will allow the evaluation of the advantages and disadvantages of the different techniques.

Data sources: We performed a systematic review using Medline, Embase and Cochrane Library electronic databases from inception of the database to September 16th 2015. Search terms included 'rotator cuff', 'scaffold', 'biomaterial', 'tissue engineering', 'cell therapy', 'platelet-rich plasma', 'growth factor'. The bibliography of each selected article was hand searched for potential further useful references that could have been missed during this process.

Study selection: Only studies focusing on *in vivo* animal models were considered and our focus was on histological and biomechanical outcomes. References that were only abstracts were rejected.

Results: A total of 77 articles were included in the review. The supraspinatus tendon of the rat is the most commonly used model followed by the infraspinatus tendon of sheep, rabbits and dogs. Small intestine submucosa (SIS) and acellular dermal matrix (ADM) scaffolds are commonly used for tendon replacement or repair reinforcement with fairly good outcomes. Matrices with continuous mineralized-demineralized transition and scaffolds made of chitin/chitosan are also promising. As for synthetic scaffolds, derivatives of polylactic acid (PLA) and polyglycolic acid (PGA) showed the best outcomes and further association of gelatin with them was shown to be beneficial. Fibroblasts, tenocytes, tendon-bone interface cells, bone marrow-derived mesenchymal stem cells (BM-MSCs), adipose-derived stem cells (ADSCs) and tendon stem/progenitor cells (TSPCs) are the cells that, once added at the repair site, gave the best outcomes, and their positive effect could be further increased by transducing the cells with important transcription factors or growth factors. The growth factors that are mostly associated with a benefit are platelet-derived growth factor BB (PDGF-BB), fibroblast growth factor 2 (FGF-2) and growth differentiation factors 5 to 7 (GDFs 5-7). The application of platelet-rich plasma (PRP) at the repair site was found to be beneficial and this effect was further increased by combination with bioactive glass powder or self-assembled peptide (SAP) gel.

Conclusions: Several materials such as SIS, ADM, PLA and PGA have beneficial effects on rotator cuff healing in animals. This is also true for cells (fibroblasts, tenocytes, tendon-bone interface cells, BM-MSCs and TSPCs), growth factors (PDGF-BB, FGF-2, GDFs 5-7) and PRP. However, there is a need to perform studies that compare these different techniques between them because it is difficult to recommend one therapy instead of another. Also further studies that investigate the combination of cell and growth factor therapy are required because until now there is little experimental data available.

Keywords: rotator cuff, scaffolds, cells, growth factors, platelet-rich plasma

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Introduction

Tendons are relatively avascular structures and their dry mass is mainly composed of collagen (65-80%) (1,2). The tendon-to-bone transition is composed of 4 zones: tendon, unmineralized fibrocartilage, mineralized fibrocartilage and bone (3,4). The three main types of collagen found in tendons are type I collagen that is abundantly found in the tendon fascicles, type II collagen that is typical for the transitional fibrocartilage, and type III collagen that is normally confined in the zones of unmineralized fibrocartilage and the endotendon (5).

Tendon disorders are very common, cause significant morbidity in everyday life and are also responsible for a non-negligible social burden (6,7). Among the musculoskeletal disorders, shoulder pain is the third most common cause after low back and cervical pain (8). Rotator cuff disorders are the most common etiology of shoulder pain (9) and several studies have shown that the prevalence and incidence of rotator cuff tears (with or without symptoms) in the general population increases with age and can even be higher than 20% (10–13). Rotator cuff lesions result either from extrinsic or intrinsic factors or a combination of both (14); common extrinsic factors are impingement (15) and acute traumatic injury, whereas intrinsic factors are generally represented by age-related degeneration. As a consequence of the possible etiologies of rotator cuff tears we can distinguish acute traumatic injuries from chronic degenerative injuries. Following a pathological examination Hashimoto *et al.* found that most injuries are chronic (16).

After an injury, tendons tend to heal spontaneously following three stages: the inflammatory, the reparative and the remodeling phases. This repair is time consuming and the result is normally scar tissue with different properties compared to normal tendon (17). The repair is similar to that seen in chronic injury (18,19) and is characterized by a decreased type I to type III collagen ratio and this correlates with poor biomechanical properties (20). This is also true for primary tendon repairs where, despite the improvements of suture techniques and post-operative rehabilitation protocols, scarring and adhesion are still frequent complications (21). For rotator cuff tears, there are many therapeutic options which begin with conservative approaches but also include more invasive surgical procedures (22–24). As for the outcome of rotator cuff repairs there are conflicting data available concerning success rates, but failure and recurrent tears are still a problem and especially for elderly patients (25–28). High age, atrophy, fatty infiltration (infiltration of the muscle by fat) and size of the tear are risk factor that predispose to retear (29–31). Although the controversial outcomes of rotator cuff repair, there is a consensus on the fact that an intact repair at follow-up provides better results than a failed one (32). All these problems combined together with population ageing show us that there is the need to develop therapeutic strategies for tendon regeneration and repair.

Different propositions have been explored to improve tendon healing. Hand surgeons already use palmaris longus and plantaris tendon autografts for flexor and extensor tendon reconstruction (33,34). Tendon autografts are also used for reconstruction of the anterior cruciate ligament (35). Biological scaffolds, in particular extracellular matrix scaffolds, are sometimes used to improve primary tendon healing in order to avoid the limited availability problems and the donor site associated complications with harvesting autograft (36). Synthetic scaffolds are other tissue engineered products that can also potentially play an important role in tendon repair (37). We will see within this review that the use of scaffolds in the shoulder is widespread. To face the extrinsic factors that lead to rotator cuff disease, decompression techniques such as acromioplasty are used

(38), whereas to face the intrinsic factors there are no established techniques. However, cell and growth factor therapies have the potential to become them (39).

The addition of cells at the injury or repair site, either by direct injection or by seeding of a carrier/matrix, can be justified by the fact that the injured tendons display an increased apoptosis rate of tenocytes (40,41) and that these cells may therefore need rapid replacement in order to re-establish normal function. The use of stem cells for the variability of tissues into which they can differentiate is particularly attractive since the healing site of a rotator cuff tendon is characterized by tendinous, cartilaginous and bony structures. BM-MSCs have the ability to differentiate into various mesenchymal tissues (42), reach the site of injury (43) and their use for tendon repair has shown promising results (44,45). Also, ADSCs are stem cells that can differentiate into various lineages and their less invasive harvesting makes them good candidates for clinical use (46–48). The isolation and characterization of TSPCs (49,50) and PPCs (51) give other sources of stem cells with the potential to be used for tendon injuries although their use is limited because of harvesting morbidity. To ameliorate the outcome obtained with cell therapies one can also transduce genes into cells in order to over-produce some proteins that could play key roles in tendon healing; among these genes we can find: scleraxis and EGR1 that are transcription factors involved in tendon development (52–55), MT1-MMP that is a membrane-bound matrix metalloproteinase implicated in formation of the tendon-bone interface (56) and growth factors.

Many growth factors, including TGF-βs, PDGF-BB, FGF-2, GDFs 5-7 and IGF-1 play an important role in the process of rotator cuff healing (57,58); GDFs 5-7 have also been found to play a role in tendon development (59). Concerning TGF-β there are 3 main isoforms: TGF-β1, TGF-β2 and TGF-β3 (60). High TGF-β3 expression is believed to be associated with the regenerative properties of fetal tissues, whereas TGF-β1 and TGF-β2 are more abundant in adults and correlate with scarring that is typically seen as less favorable (61–63). G-CSF is another growth factor that can find its application due to its role in inflammation and chemotaxis of mesenchymal stem cells (64,65). A simple injection of growth factors at the repair site is probably not sufficient to significantly stimulate the healing process because of clearance; in order to extend the period in which growth factors have an effect they can by delivered via osmotic pumps (66), collagenous matrices (67), hydrogels (68), electrospun scaffolds (69), SAP (70) among others. In order to increase growth factor expression they can also be transduced into cells (71).

Platelet-rich plasma (PRP) is a fraction of plasma highly concentrated with platelets that is also an interesting substance in tendon regeneration since platelets contain growth factors important for tendon healing such as TGF-βs, PDGFs, FGF-2 and IGF-1 (72,73). Additionally, the use of PRP has been seen to promote differentiation of TSPCs into tenocytes (74).

Rotator cuff repair is a field where tissue engineering, a discipline that studies the combination of cells, scaffolds, growth factors and other biochemical factors for tissue regeneration, has already found its application on humans but these techniques are still not part of surgical routine (75–86).

In order to have a better overview of the different techniques, we will base our observations using the following definitions:

- *Scaffold Interposition* (Figure 1A): the scaffold plays a central mechanical role by being interposed between tendon and bone or between two tendon stumps.
- *Augmentation*: the scaffold or another material reinforces the repair or is placed in a critical location where it could stimulate healing (the mechanical aspect is secondary). There are two types of augmentation:

- *Overlapping of the repair site* (Figure 1B): clear mechanical reinforcement of the repair by overlapping a scaffold over the repair site.
- *Filling of the repair site* (Figure 1C): the material is applied in order to fill a space (often the tendon-bone interface or a bone defect).

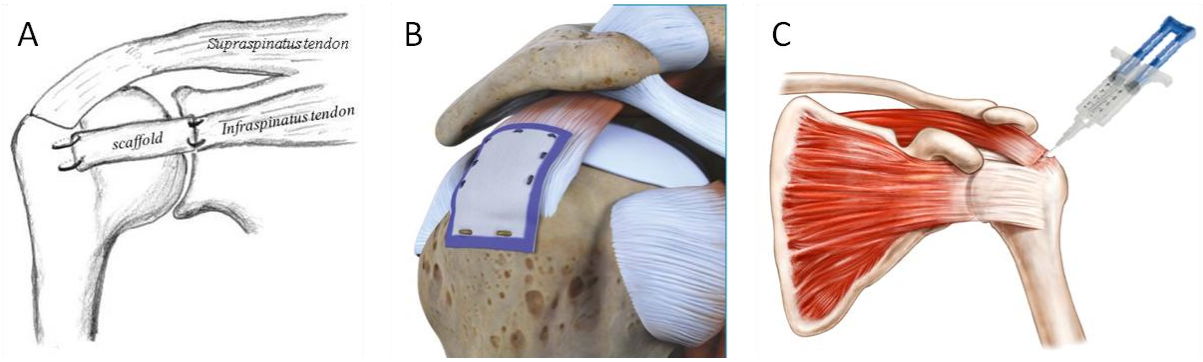


Figure 1. Augmentation techniques for rotator cuff

A) Scaffold interposition (figure from: Inui *et al.* Application of layered poly (L-lactic acid) cell free scaffold in a rabbit rotator cuff defect model. Sport Med Arthrosc Rehabil Ther Technol. BioMed Central Ltd). B) Scaffold overlapping (Figure from: http://www.howardluksmd.com). C) Biomaterial filling (adapted from: http://shouldercenter.org).

This review aims to evaluate the literature concerning the materials, cells and growth factors used for rotator cuff repair *in vivo* on animal models, in order to elucidate the advantages and disadvantages of the different techniques with focus on biomechanical and histological outcomes. There are many articles reviewing these different techniques especially on humans (37,75–94), but to our knowledge none of them have analyzed all these techniques together when used on animals in a systematic way. Animal testing presents important pre-clinical work and allows a sufficiently large population in a controlled environment. Furthermore, in the field of tissue engineering where novel therapeutic strategies are continuously being developed, pre-clinical research is essential. This research should help to identify possible new therapies to apply on humans. The questions we will try to answer are as follows:

- ***Which materials are currently available for rotator cuff interposition and repair site overlapping in animals and what are their main advantages and disadvantages?***
- ***Which materials are currently available for rotator cuff repair filling in animals and what are their main advantages and disadvantages?***
- ***Does the addition of cells and/or growth factors improve the results?***
- ***Does the use of PRP improve the results?***
- ***Which of these methods are the most promising and have the potential to become useful on humans?***

Methods

Eligibility criteria

Only studies focusing on animals were considered. Our PICOS criteria for systematic reviews are:

- *Participants*: *in vivo* animal models
- *Interventions*: scaffolds, cells and/or growth factors for rotator cuff repair with local delivery
- *Comparisons*: absence of augmentation/interposition and/or absence of cells and/or absence of growth factors
- *Outcomes*: histological and/or biomechanical results

- *Study design*: all types of articles

No limits were established for year of publication and references in English, French, German and Italian were considered.

Search strategy

The useful studies were identified and all that were within Medline, Embase and Cochrane Library electronic databases were consulted. The last search was performed on September 16th 2015. The following search terms were used: 'rotator cuff', 'scaffold', 'biomaterial', 'tissue engineering', 'cell therapy', 'platelet-rich plasma', 'growth factor'. In the appendix the full search strategy for the databases is presented and for the selection of the useful references we read the title and abstract of each of the articles.

In addition, the bibliography of each selected article was hand searched for potential further useful references that could have been missed, but which would fulfill the eligibility criteria.

Data collection process

In order to extract the relevant data from the selected articles, we developed an Excel sheet in which we included all the important information such as type of tendon, animal model, number of participants, type of injury, repair technique, scaffold, cells, growth factors, biomechanical outcomes, histological outcomes and further relevant data.

Method of analysis

The therapies (including controls) were separated and analyzed in 7 categories:

1. Scaffold/Biomaterial interposition
2. Scaffold/Biomaterial overlapping the repair site
3. Scaffold/Biomaterial filling the repair site
4. Cells
5. Growth factors
6. Cells and growth factors
7. PRP

The first 3 categories included all the studies that tested a material respectively for interposition, overlapping or filling without any other supplemental treatment such as cells or growth factors. Categories 4 and 5 included studies in which cells and growth factors, respectively were administered alone or via a carrier. The studies in which cells and growth factors were given together are in category 6, whereas category 7 included all the studies in which the use of PRP was reported. It is important to note that we decided to put PRP in an additional and separate category and not to put it into categories 4, 5 or 6 since there are many studies reporting its use and there is no consensus whether to consider it a growth factor or a cell therapy. In addition, we also report that some studies used transduction of growth factors into cells (these studies are analyzed in category 6). Articles that reported the use of transduced cells with genes other than growth factors are in category 4.

For each category the outcomes of the different studies are summarized in a table that also reports authors and reference, animal model, tendon studied (SS = Supraspinatus, IS = Infraspinatus, SSc = Subscapularis, NS = not specified), type of injury (acute or chronic) and type of analysis (B = Biomechanics, H = Histology). We do not report the numerical biomechanical data because there is too much variation among the reported studies with regard to measurement techniques, animal models and type of tendons studied and therefore it is difficult to make comparisons based on numbers. Furthermore, many studies reported biomechanical outcomes for load-at-failure (in

Newtons), deformation (in millimeters) and stiffness (in N/mm) that are parameters that depend on the size of the "product/samples" which is also different among studies.

Results

Study selection

A total of 77 articles were included for the establishment of the final review. Due to the use of the extensive search equations, a total of 530 references were found in the 3 databases, and 83 of them were accepted with relation to title and abstract reading. Ten references had to be excluded because they were only conference abstracts and did not provide exhaustive information. Additional 4 articles were included when hand search of the bibliographies of the selected articles was accomplished. The details of the selection are provided in Figure 2.

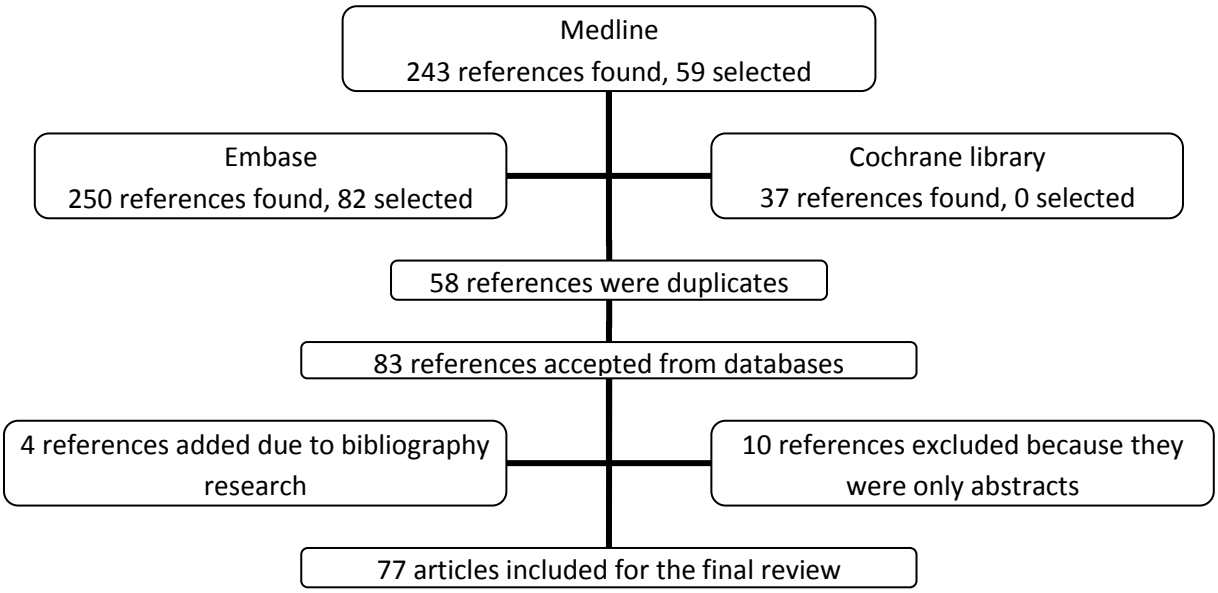


Figure 2. Flow diagram of study selection

The reasons for exclusion were for many reasons clear such as: absence of *in vivo* testing, use of cadaveric models, use of therapies that were outside our criteria, use of systemic therapies (only local therapies were included), articles in languages other than English, French, German or Italian. Studies in which the authors considered an autograft as a scaffold (e.g. use of the patellar tendon for supraspinatus tendon repair) were also excluded because we wanted to remain in the tissue engineering field. Furthermore, we decided to reject references that were only abstracts because the limited information they delivered could lead towards a positive outcome.

Characteristics of included studies

The number of articles published for each year are illustrated in Figure 3. We can observe a clear trend towards augmentation of publications in this special domain.

The rat model has a total of 42 publications and has been the most used animal model, followed by the rabbit with 15, sheep with 11, dog with 7 and goat and monkey both with 1. When looking at the tendons that are most studied, it is clear that the supraspinatus and the infraspinatus tendons are the most studied. In particular, the supraspinatus of the rat plays the major role, followed by the infraspinatus of sheep, rabbit and dog and details are provided in Figure 4.

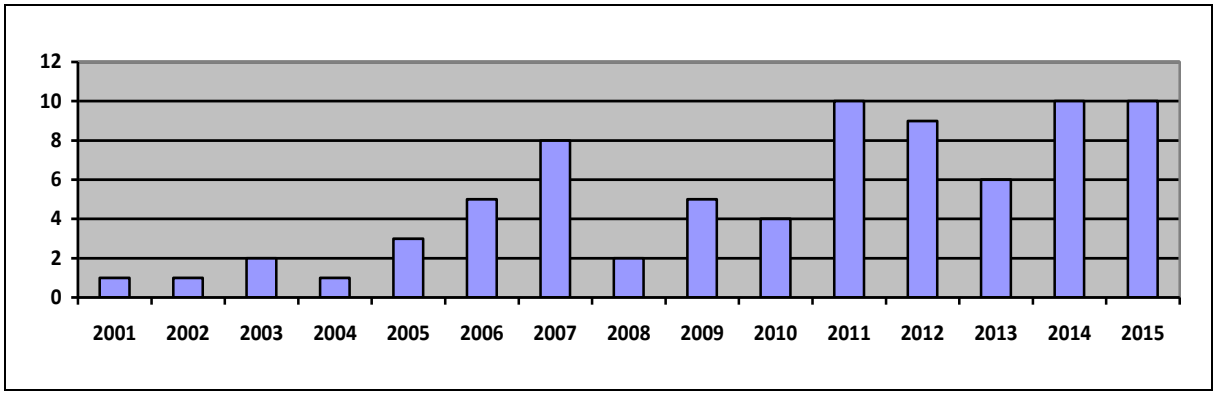


Figure 3. Number of articles per year

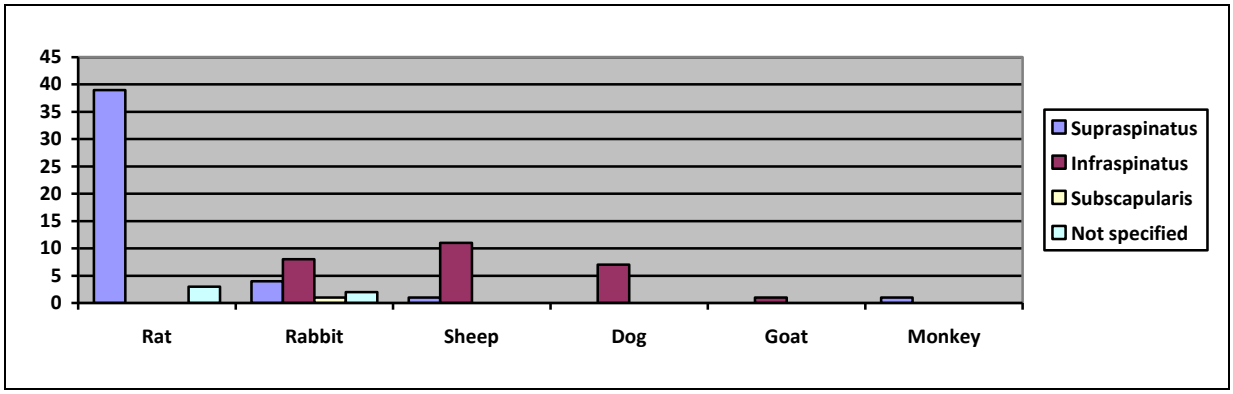


Figure 4. Animal model and type of tendon studied

Two large categories that we would like to differentiate are the acute and chronic injury models. Sixty-two articles reported experiments on acute injury models, whereas 16 of them report experimentation in a chronic scenario. Of the 62 chronic models 33 were with rats, 12 with rabbits, 9 with sheep, 6 with dogs, 1 each was for a goat and a monkey. Of the 16 chronic models 10 were with rats, 3 with rabbits, 2 with sheep and 1 with a dog.

Several articles reported more than one type of therapy that were accepted and reported in this review. For example there were studies concerning the filling of the repair site with a biomaterial alone and filling of the repair site with the same biomaterial loaded with cells or growth factors. As a consequence the number of therapies analyzed is larger than the total number of articles included in the review.

In vivo outcomes

1. Scaffold interposition

Studies reporting scaffold interposition are summarized in Table 1.

BIOLOGICAL SCAFFOLDS

We have identified 5 studies that used SIS as an interpositional graft. Dejardin *et al.* (95) found that the use of SIS resulted in a weaker repair compared to primary suture of the tendon immediately postoperative, whereas later on the augmentation became biomechanically and histologically equivalent. Zheng *et* al. (96) showed that SIS implantation was inferior to the use of autologous tissue because it elicited an inflammatory reaction and the tissue organization was weaker. In the context of a tendon midsubstance defect, Zalavras *et al.* (97) observed that SIS interposition resulted in a stronger and more organized tendon after 16 weeks compared to injury without repair. In a comparison with autologous tissue interposition, Chen *et al.* (98) found that the use of SIS was

Table 1. Scaffold interposition

Authors	Model	Tendon	Injury	Device	Analysis	Outcomes
			Biological scaffolds			
Dejardin *et al.* (95)	Dog	IS	Acute	SIS	B,H	Weaker repair immediately postoperative compared to primary suture. SIS resorbed after 12w.
Zheng *et al.* (96)	Rabbit	SS	Acute	SIS Restore™	H	More inflammation and less fiber organization compared to autologous tissue.
Zalavras *et al.* (97)	Rat	SS	Acute	SIS Restore™	B,H	Stronger repair after 16w and better tissue organization compared to no repair.
Chen *et al.* (98)	Rabbit	NS	Acute	SIS Restore™	H	Similar to autologous tissue after 8w but ectopic bone in the tendon and more inflammation.
Perry *et al.* (99)	Rat	SS	Acute, chronic	SIS	B,H	Similar to no augmentation after 16w. Smaller cross-sectional area in the chronic model.
Adams *et al.* (100)	Dog	IS	Acute	ADM Graftjacket®	B,H	Similar to autologous tissue but increased inflammation after 12 and 24w. ADM not fully resorbed.
Ide *et al.* (101)	Rat	SS	Acute	ADM Graftjacket®	B,H	Better load-at-failure and histology after 2, 6 and 12w than no repair. ADM resorbed after 6w.
Ide *et al.* (102)	Rat	SS	Acute	ADM Graftjacket® and Fibrin sealant fill.	B,H	See later growth factor addition.
Xu *et al.* (103)	Monkey	SS	Acute	ADM Conexa®	H	Graft invaded by fibroblasts and low inflammation after 12w; further tissue organization after 24w.
Uezono *et al.* (104)	Rat	SS	Acute	ADM Graftjacket®	B,H	Increased load-at-failure, fibrocartilage formation and tissue organization with 2w of immobilization.
Chen *et al.* (98)	Rabbit	NS	Acute	Type I/III collagen ACI-Maix™	H	Similar to autologous tissue after 8w but ectopic adipose tissue in the tendon and more inflammation.
Roßbach *et al.* (105)	Sheep	IS	Acute	Type I collagen	B	Weaker than normal tendon after 12w. See cell seeding below.
Shen *et al.* (106)	Rabbit	NS	Acute	Silk-type I collagen	B,H	See cell seeding below.
Dickerson *et al.* (107)	Sheep	SS and IS	Acute	Bovine bone	H	Organized tissue and fibrocartilage deposit as opposed to standard repair. No inflammation after 16w.
Funakoshi *et al.* (108)	Rabbit	IS	Acute	Chitosan-based HA	B,H	Increased load-at-failure and Young's modulus compared to no repair after 4 and 12w. In part absorbed after 12w but disorganized tissue.
Funakoshi *et al.* (109)	Rabbit	IS	Acute	Chitin fabric	B,H	Better load-at-failure, stiffness and histology than no repair after 12w with partial resorption.
Thomopoulos *et al.* (110)	Rat	SS	Acute	Fibrin clot	B,H	Weaker repair and no histological changes after 3w compared to sutures. Resorption after 12w.
			Synthetic scaffolds			
Aoki *et al.* (111)	Dog	IS	Acute	PLLA	B,H	Progressive increase in strength, PLLA tolerated, sparse connection with bone. Partial absorption after 32w.
Inui *et al.* (112)	Rabbit	IS	Acute	PLLA	B,H	Similar biomechanical properties as suture and normal values after 8w. Absorption began at 16w.
Kim *et al.* (113)	Rabbit	IS	Acute	PLA	H	Foreign-body reaction that peaked after 4w and decreased afterwards. See cell seeding below.
Inui *et al.* (114)	Rabbit	IS	Acute	PLG	B,H	Inferior load-at-failure than primary suture after 0 and 4w. Healing delayed. Resorption after 8w.
Yokoyka *et al.* (115)	Rabbit	IS	Acute	PGA	B,H	Stronger compared to no repair, formation of fibrocartilage interface and absorption after 16w.
Yokoya *et al.* (116)	Rabbit	IS	Acute	PLC or PGA	B,H	PLC inferior to PGA on biomechanics and histology: foreign-body reaction (not with PGA), fibers present after 16w (not with PGA), less organization.
Kimura *et al.* (117)	Dog	IS	Acute	PTFE Teflon®	B,H	Progressive increase in biomechanical and histological properties over 12w. Foreign body reaction at the margins of the scaffold.

inferior because it was characterized by induction of ectopic bone in the tendon and increased inflammation. Perry *et al.* (99) tested SIS in both an acute and a chronic injury model; in both cases there was no biomechanical and histological benefit compared to primary repair after 16 weeks but

it was interesting to note that the augmented chronic model healed with a smaller cross-sectional area.

In 4 studies the authors interposed ADM for tendon repair. Adams *et al.* (100) showed that interposition with ADM gave similar biomechanical and histological outcomes as autologous tissue; ADM implantation was however characterized by increased inflammation after 3 and 6 months and the scaffold was not fully absorbed at that time. In the context of a supraspinatus tendon resection, Ide *et al.* (101) found that ADM interposition resulted in increased load-at-failure, better tendon organization and more fibrocartilage deposition after 2, 6 and 12 weeks compared to no repair; the ADM graft was fully resorbed after 6 weeks. The only primate experimentation reported in this review was performed by Xu *et al.* (103) that interposed ADM and found that the graft elicited a low degree inflammation and allowed the formation of a tendon-like tissue after 6 months. In a study focusing on the effects of postoperative immobilization in the context of ADM interposition in rats, Uezono *et al.* (104) found that two weeks of immobilization allowed an increase in load-at-failure, better fibrocartilage formation, and higher tissue organization after 12 weeks compared to no immobilization or longer periods of immobilization.

Three other studies employed other types of collagen-based scaffolds. Chen *et al.* (98) found that the use of a type I/III collagen scaffold was inferior to autologous tissue because it was associated with induction of adipose tissue in the tendon and increased inflammation after 8 weeks. In two other studies, Roßbach *et al.* (105) and Shen *et al.* (106) used a type I collagen scaffold and a silk-type I collagen sponge respectively, but their aim was to study the impact of cell seeding on these scaffolds and we will address this later because no other treatment was present as a comparison. Roßbach *et al.* reported that the use of the type I collagen scaffold did not allow to attain normal tendon load-at-failure after 12 weeks.

There are also other biological scaffolds that were used for interposition in animal models. Dickerson *et al.* (107) reported that the use of a biphasic scaffold with a continuous hard tissue (mineralized)-soft tissue (demineralized) transition derived from bovine cancellous bone allowed clear histological improvements compared to standard transosseous repair such as induction of a more organized tissue and presence of typical insertional fibrocartilage after 16 weeks in the absence of inflammation. Funakoshi *et al.* compared the interposition of either chitosan-based HA (108) or chitin (109) with a resection without repair; both scaffolds allowed an increase in biomechanical and histological properties during 12 weeks and no foreign-body reaction was reported with the scaffolds being only partially resorbed after 12 weeks. By interposing a fibrin clot within a tendon defect and comparing it with simple sutures, Thomopoulos *et al.* (110) found that the fibrin clot was associated with reduced biomechanical properties (tensile strength and Young's modulus) after 3 weeks and no histological changes; the clot was completely resorbed after 12 weeks.

SYNTHETIC SCAFFOLDS

Aoki *et al.* (111) observed that PLLA interposition allowed a progressive increase in load-at-failure and stiffness, together with formation of a good connection with the tendon but a limited one with the bone; there were no signs of foreign-body reaction and the PLLA fibers began to be absorbed after 32 weeks. In a comparison with primary suture of the tendon, Inui *et al.* (112) found that PLLA interposition was biomechanically equivalent to primary suture and reached normal load-at-failure and stiffness values after 8 weeks; the PLLA was progressively invaded by fibrous tissue and began to be resorbed after 16 weeks. Kim *et al.* (113) reported that the use of an open-cell PLA scaffold elicited a foreign body reaction that peaked after 4 weeks and decreased thereafter; the aim of this

study was to observe the effect of cell seeding so that more details are given under cell seeding below.

A PLG scaffold was used by Inui *et al.* (114) who found that load-at-failure was inferior compared to primary suture immediately postoperative and after 4 weeks but was similar thereafter. In addition, the histology revealed that healing was a little bit delayed over the 16 week period of observation.

The interposition of a PGA scaffold by Yokoya *et al.* (115) allowed to obtain better biomechanical properties and formation of a fibrocartilage tendon-bone interface compared to resection without repair; the graft was completely absorbed after 16 weeks. The same study group (116) compared PLC and PGA scaffolds and it was shown that PGA scaffolds were better for both biomechanical and histological outcomes. In fact PGA was characterized by increased load-at-failure and Young's modulus after 4 and 16 weeks, by the absence of a foreign-body reaction and by stimulation of a more organized tissue.

Kimura *et al.* (117) used PTFE and observed a progressive increase in biomechanical and histological properties over 12 weeks. However, there was evidence of a foreign-body reaction at the margins of the scaffold.

2. Scaffold overlapping the repair site

Studies reporting scaffold overlapping are summarized in Table 2.

BIOLOGICAL SCAFFOLDS

Two articles reported the use of SIS to overlap the repair site. Schlegel *et al.* (118) found that SIS augmentation allowed an increase in stiffness compared to repair without augmentation, whereas on histology no improvement was observed but the SIS patch was well tolerated by the host. On the other hand, Nicholson *et al.* (119) found that SIS did not result in increased biomechanical properties but there was histological improvement represented by decreased necrosis around the sutures and there was induction of ectopic bone.

Only Smith *et al.* (120) reported the use of ADM in the context of overlapping: in a chronic scenario repair, use of ADM was equivalent to debridement for both biomechanical and histological outcomes after 6 months even though the graft was surrounded by an inflammatory infiltrate.

In four additional studies there was the use of collagen-based scaffolds. Nicholson *et al.* (119) found that a PD patch did not result in increased biomechanical properties but, similar to SIS, it decreased necrosis around the sutures. As opposed to SIS, it did not induce ectopic bone but it was less integrated with the host tissue after 9 weeks. In another study, Chung *et al.* (121) found that a similar PD patch did not change the biomechanical and histological outcomes during 8 weeks after repair. Kovacevic *et al.* (122) used a type I collagen patch and observed that it reduced load-at-failure and stiffness and in addition it did not change the histological outcomes after 4 weeks compared to no augmentation. Another outcome showing no advantages was found by Peterson *et al.* (123) who used a P4HB/type 1 bovine collagen scaffold and observed that no histological benefit was provided.

SYNTHETIC SCAFFOLDS

In the context of PLA overlapping, MacGillivray *et al.* (124) found that this technique had no biomechanical and histological advantages compared with no augmentation; the PLA was not absorbed after 24 weeks and elicited a foreign-body reaction. Derwin *et al.* (125) used a PLLA patch and found on the contrary that its use was beneficial since it increased load-at-failure and stiffness of the repair and was also biocompatible after 12 weeks. A PLLA patch was also used by Zhao *et al.* (126) and they found also that it allowed an increase in load-at-failure and positive histological

Table 2. Scaffold overlapping

Author	Model	Tendon	Injury	Device	Analysis	Outcomes
				Biological scaffolds		
Schlegel *et al.* (118)	Sheep	IS	Acute	SIS CuffPatch™	B,H	Increased stiffness, no histological advantage compared to no augmentation and no foreign-body reaction. Partial absorption after 12w.
Nicholson et *al.* (119)	Sheep	IS	Acute	SIS Restore™	B,H	No biomechanical changes but less necrosis and ectopic bone compared to no augmentation.
Smith *et al.* (120)	Dog	IS	Chronic	ADM Graftjacket®	B,H	No changes compared to debridement after 24w. Lymphocytic infiltrate around the graft.
Nicholson *et al.* (119)	Sheep	IS	Acute	PD Zimmer®	B,H	No biomechanical changes but less necrosis compared to no augmentation. Less integrated than SIS after 9w.
Chung *et al.* (121)	Rabbit	SS	Chronic	PD Permacol™	B,H	No changes compared to no augmentation.
Kovacevic *et al.* (122)	Rat	SS	Acute	Type I collagen BioBlanket™	B,H	Biomechanically detrimental and histologically not relevant compared to no augmentation after 4w.
Peterson *et al.* (123)	Sheep	IS	Acute	P4HB/ Type I collagen BioFiber™-CM	H	No changes after 8w compared to no augmentation.
				Synthetic scaffolds		
MacGillivray *et al.* (124)	Goat	IS	Acute	PLA	B,H	No advantages compared to no augmentation. PLA not absorbed after 24w and little foreign-body reaction.
Derwin *et al.* (125)	Dog	IS	Acute	PLLA X-Repair	B,H	Increased load-at-failure and stiffness during 12w compared to no augmentation. Good biocompatibility.
Zhao *et al.* (126)	Rat	SS	Chronic	PLLA or PLLA/ gelatin	B,H	Best outcomes for PLLA/gelatin but PLLA was also superior compared to no augmentation. Absorption after 8w.
Baker *et al.* (127)	Dog	IS	Acute	Fascia/PLLA	B,H	Biomechanical advantage immediate after repair but disadvantage after 12w. Patch not resorbed after 12w. No foreign-body reaction.
Taylor *et al.* (128)	Rat	SS	Acute	PLAGA	B	Higher Young's modulus compared to no augmentation after 8w.
Zhao *et al.* (129)	Rat	SS	Chronic	PLGA	B,H	Increased load-at-failure, stiffness, fibrocartilage and organization during 8w. Absorption after 8w.
Dines *et al.* (130)	Rat	NS	Chronic	PGA	B,H	No advantages compared to no augmentation after 6w.
Santoni *et al.* (131)	Sheep	IS	Acute	PU	B,H	Increased load-at-failure after 12w compared to no augmentation. No foreign-body reaction.
Cole *et al.* (132)	Rat	SS	Acute	PC-PE	H	Better collagen alignment compared to no augmentation and no foreign-body reaction after 6w.
Beason *et al.* (133)	Rat	SS	Acute	PCL or PCL/PEO	B,H	No advantages compared to no augmentation. PCL better infiltrated than PCL/PEO.

properties such as better fibrocartilage formation and collagen organization. Additionally, the authors also studied the effect of grafting gelatin on the PLLA membrane and they found that this was even more beneficial than PLLA alone with further increased biomechanical properties and more cartilage deposition and collagen organization. Baker *et al.* (127) reported that the use of a reinforced fascia patch composed of human fascia lata and PLLA (fascia/PLLA) increased load-at-failure immediately postoperative but decreased after 12 weeks. The scaffold was not resorbed after 12 weeks and it did not elicit a foreign-body reaction.

A PLAGA scaffold, studied by Taylor *et al.* (128), allowed an increase in Young's modulus compared to no augmentation after 8 weeks and a PLGA scaffold, used by Zhao *et al.* (129), allowed a significant increase in load-at-failure and stiffness after 4 weeks together with better fibrocartilage formation and collagen organization during 8 weeks.

Dines *et al.* (130) found that overlapping with PGA did not result in histological or biomechanical changes after 6 weeks compared to no augmentation.

Two groups studied the effects of PU-based scaffolds. Santoni *et al.* (131) found that PU provided a higher load-at-failure for the repair after 12 weeks and that PU was well integrated with the host tissue without causing an immunological response. Similarly Cole *et al.* (132) observed that a PC-PE patch was well tolerated by the host and also improved collagen fiber alignment after 6 weeks.

Beason *et al.* (133) overlapped the repair site with either PCL or PCL/PEO and found that both did not provide biomechanical and histological advantages compared to no augmentation. The PCL scaffold showed better integration with the host tissue than PCL/PEO by allowing more cellular infiltration.

3. Scaffold/Biomaterial filling the repair site

Studies reporting filling of the repair site with materials are summarized in Table 3.

Table 3. Scaffold/biomaterial filling the repair site

Author	Model	Tendon	Injury	Device	Analysis	Outcomes
				Biological materials		
Rodeo *et al.* (134)	Sheep	IS	Acute	Type I collagen ReGen	B,H	No advantages compared to no augmentation. Complete resorption after 6w.
Seeherman *et al.* (135)	Sheep	IS	Acute	Type I/III collagen	B,H	No advantages compared to no augmentation.
Hee *et al.* (136)	Sheep	IS	Acute	Type I collagen	B,H	No advantages compared to no augmentation.
Murray *et al.* (137)	Rat	SS	Acute	Collagen Helistat®	B,H	See growth factor addition below.
Mora *et al.* (138)	Rat	SS	Acute	Collagen	B,H	See cell seeding below.
Loeffler *et al.* (139)	Rat	SS	Acute	Gelfoam®	H	Increased inflammation after 3w.
Tokunaga *et al.* (140)	Rat	SS	Acute	Gelatin hydrogel MedGEL PI5	B,H	No advantages compared to no augmentation.
Gulotta *et al.* (141)	Rat	SS	Acute	Fibrin sealant Tisseel®	B,H	No advantages compared to no augmentation.
Ide *et al.* (142)	Rat	SS	Acute	Fibrin sealant	B,H	See growth factor addition below.
Cheng *et al.* (143)	Rat	SS	Acute	Fibrin sealant	B	See cell seeding below.
Li *et al.* (144)	Rat	SS	Acute	Fibrin sealant Tisseel®	B,H	See cell seeding below.
Manning *et al.* (145)	Rat	SS	Acute	HBDS	B,H	Increased load-at-failure and no histological changes compared to no augmentation after 8w.
Kovacevic *et al.* (146)	Rat	SS	Acute	Ca-P	B,H	No biomechanical advantage but more cartilage, better organization and higher collagen I/III ratio compared to no augmentation after 2w.
Zhao *et al.* (147)	Rat	SS	Chronic	CPS or HA ceramic powder	B,H	Increased properties after 4 and 8w compared to no augmentation. CPS superior to HA.
Buchmann *et al.* (148)	Rat	SS	Chronic	VPG	B,H	No advantages compared to no augmentation.
Kim *et al.* (149)	Rat	SS	Chronic	SAP KLD-12	H	No advantages compared to saline.
				Synthetic materials		
Chen *et al.* (150)	Rabbit	IS	Acute	PEGDA	B,H	See cells seeding and growth factor addition below.
Moffat *et al.* (151)	Rat	SS	Acute	PLGA/ PLGA-HA	H	Better fibrocartilage formation and organization after 5w compared to no augmentation.

The addition of collagen-based materials and gelatins alone at the repair site (e.g. at the tendon-bone interface) was never found to be beneficial compared to no augmentation (134–136,139,140) and in one case it even resulted in increased inflammation after 3 weeks (139).

Gulotta *et al.* (141) added fibrin sealant between the tendon and bone and found that it did not change the outcome after 4 weeks. Some improvements were found by Manning *et al.* (145) who used HBDS gel which allowed an increase in load-at-failure after 8 weeks.

Kovacevic *et al.* (146) added a Ca-P matrix at the repaired tendon footprint and found that it did not change neither load-at-failure nor stiffness during 4 weeks, whereas after 2 weeks Ca-P presence featured more cartilage deposition, increased collagen organization and increased collagen I/III ratio. In a similar manner, Zhao *et al.* (147) added either CPS or HA ceramic powders at the repaired tendon footprint and observed that both bioceramics allowed an increase in load-at-failure and stiffness after 4 and 8 weeks compared to no augmentation but CPS was clearly better than HA after 8 weeks. Furthermore, histology for the two bioceramics improved the overall outcome with more bone ingrowth, cartilage deposition and collagen organization and CPS was better since it was characterized by more fibrocartilage deposition. The addition of VPG at the repair site by Buchmann *et al.*(148) and the addition of SAP at the injury site by Kim *et al.* (149) did not change the outcomes after 6 and 5 weeks, respectively.

Moffat *et al.* (151) added, between the tendon and bone, a biphasic scaffold with contiguous non-mineralized (PLGA) and mineralized (PLGA-HA) regions that allowed an improvement in fibrocartilage formation and organization after 5 weeks compared to no augmentation.

4. Cells

The use of cell therapy is summarized in Table 4.

Several types of fibroblastic cells have been reported to have been added at the repair site. Funakoshi *et al.* (108) seeded a chitosan-based HA scaffold with fibroblasts and found that the cells boosted the positive effect that was already cited for the scaffold by further increasing the biomechanical properties and by improving collagen organization. In two studies the authors used tenocytes; the addition of the cells by Chen *et al.* (98) to a type I/III collagen scaffold or a SIS scaffold used as interpositional grafts allowed an improvement in histological features such as a reduction in inflammation and more type I collagen deposition. The presence of tenocytes also allowed a reduction in the ectopic adipogenesis observed in animals receiving the type I/III collagen scaffold alone, whereas there was no reduction in the ectopic bone formation observed for the SIS scaffold. Roßbach *et al.* (105) seeded a type I collagen scaffold with tenocytes and this was used for interposition where they found that the presence of the cells allowed an increase in biomechanical properties so that after 12 weeks load-at-failure became similar to normal tendon. Addition of tendon-bone interface cells in a piece of Gelfoam®, reported by Loeffler *et al.*(139), resulted in accelerated healing with better collagen organization after 12 weeks. We will see later that two studies used fibroblasts in concomitance with growth factor therapy.

Pelinkovic *et al.* (152) injected MDCs at the injury site and found that after 3 weeks the cells could differentiate into tenocyte-like cells and that after this period the tendon fibers were realigned. However, comparison with other treatments was not performed and thus we cannot say if the use of MDCs is advantageous.

The use of BM-MSCs is very common in the literature; Yokoya *et al.* (115) found that addition of these cells into a PGA device used for interposition further increased the biomechanical benefits

Table 4. Cell therapy

Author	Model	Tendon	Injury	Cells	Scenario	Analysis	Outcomes
Funakoshi *et al.* (108)	Rabbit	IS	Acute	Fibroblasts	Chitosan-based HA interpos.	B,H	Increased load at failure, Young's modulus and collagen alignment after 12w compared to no cells.
Chen *et al.* (98)	Rabbit	NS	Acute	Tenocytes	Type I/III collagen interpos.	H	Less inflammation, less ectopic adipogenesis, better absorption and more type I collagen than no cells.
Chen *et al.* (98)	Rabbit	NS	Acute	Tenocytes	SIS interpos.	H	Less inflammation, same ectopic osteogenesis, better absorption and more type I collagen than no cells.
Roßbach *et al.* (105)	Sheep	IS	Acute	Tenocytes	Type I collagen interpos.	B	Increased load-at-failure compared to no cells after 12w.
Loeffler *et al.* (139)	Rat	SS	Acute	Tendon-bone interface cells	Gelfoam® fill.	H	Better than no cells after 12w.
Pelinkovic *et al.* (152)	Rat	SS	Acute cryoinjury	MDCs	Cells injection	H	Differentiation into tenocyte-like cells, tendon fibers realigned after 3w.
Yokoyka *et al.* (115)	Rabbit	IS	Acute	BM-MSCs	PGA interpos.	B,H	Better load-at-failure, Young's modulus, Sharpey's fibers and type I collagen than no cells after 16w.
Kim *et al.* (113)	Rabbit	IS	Acute	BM-MSCs	PLA interpos.	H	More type I collagen and organization after 6w compared to no cells.
Gulotta *et al.* (141,153–155)	Rat	SS	Acute	BM-MSCs	Fibrin sealant fill.	B,H	No advantages compared to no cells and no augmentation.
Gulotta *et al.* (153)	Rat	SS	Acute	BM-MSCs MT1-MMP-transduced	Fibrin sealant fill.	B,H	Increased load-at-failure, stiffness and fibrocartilage compared to BM-MSCs after 4w.
Gulotta *et al.* (154)	Rat	SS	Acute	BM-MSCs scx-transduced	Fibrin sealant fill.	B,H	Increased load-at-failure, stiffness and fibrocartilage compared to BM-MSCs after 4w (stiffness at 2w too).
Li *et al.* (144)	Rat	SS	Acute	BM-MSCs and BM-MSCs TGIF1 silenced	Fibrin sealant fill.	B,H	Increased load-at-failure, stiffness and fibrocartilage for TGIF1-silenced compared to BM-MSCs after 4w.
Oh *et al.* (156)	Rabbit	SSc	Chronic	ADSCs	Cells injection	B,H	No biomechanical change but less fatty infiltration than saline after 6 weeks.
Chen *et al.* (157)	Rat	SS	Acute collagenase	ADSCs	Cells injection	B,H	Increased load-at-failure after 1 week, faster healing than PBS during 3 weeks.
Mora *et al.* (138)	Rat	SS	Acute	ADSCs	Collagen fill.	B,H	No biomechanical advantage, less inflammation at 1w, less fibrocartilage at 4w compared to no cells.
Shen *et al.* (106)	Rabbit	NS	Acute	TSPCs	Silk-type I collagen interpos.	B,H	Better biomechanical properties, more collagen and less inflammation than no cells after 12 weeks.
Cheng *et al.* (143)	Rat	SS	Acute	TSPCs and TSPCs TSG-6 silenced	Fibrin sealant fill.	B	Increased biomechanical properties for normal TSPCs compared to no cells and TSG-6 silenced TSPCs after 4w.
Tao *et al.* (158)	Rabbit	IS	Chronic	TSPCs and TSPCs EGR1-transduced	Fibrin sealant fill.	H	More density and Sharpey's fibers with cells. More Sharpey's and collagen I with EGR1 transduction after 8w.
Gumucio *et al.* (159)	Rat	SS	Chronic	SVFCs	Cells injection	H	Reduction in muscle fibrosis compared to Ringer's after 2 weeks.

already provided by the scaffold after 16 weeks together with increased Sharpey's fiber (collagen fibers that extend from tendons into the bones to which they attach) formation and type I collagen deposition. In the context of a seeded PLA graft used for interposition Kim *et al.* (113) found that BM-MSCs stimulated more type I collagen deposition and increased tissue organization after 6 weeks.

Gulotta *et al.* (141) added BM-MSCs to fibrin sealant administrated at the repair site and found that fibrin sealant with or without cells did not change the biomechanical and histological properties of

the repair after 4 weeks. Other studies by the same authors (153–155) used the same BM-MSC treatment as a control for further investigations such as transduction of the stem cells with MT1-MMP (153) and scleraxis (154) which resulted in increased load-at-failure, stiffness and fibrocartilage formation compared to normal BM-MSCs after 4 weeks and scleraxis increased stiffness after 2 weeks too. Li *et al.* (144) worked also with modified BM-MSCs delivered via fibrin sealant at the repair site, and found that silencing TGIF1 increased load-at-failure, stiffness and fibrocartilage formation compared to normal BM-MSCs after 4 weeks.

Oh *et al.* (156) found that ADSC injection at the repair site did not change load-at-failure but resulted in less fatty infiltration compared to saline injection after 6 weeks. An ADSC injection was also performed by Chen *et al.*(157) who added the cells at the injury site and noticed that they were associated with higher load-at-failure after 1 week and more rapid histological healing during 3 weeks compared to PBS injection. Mora *et al.* (138) found that seeding of a collagen carrier added at the repair site with ADSCs did not change neither load-at-failure nor stiffness after 4 weeks compared with no cells, whereas on histology analysis the presence of ADSCs correlated at the beginning with less inflammation and afterwards it was also characterized by less fibrocartilage deposition.

Other stem cells used in this domain are TSPCs. Shen *et al.* (106) interposed seeded silk-type I collagen and found that TSPCs increased stiffness, Young's modulus and collagen deposition together with reducing inflammation during 12 weeks compared to no cells. Cheng *et al.* (143) seeded fibrin sealant that was added at the tendon-bone interface with either normal TSPCs or with TSG-6 silenced TSPCs. Biomechanical analysis revealed that normal TSPCs increased tensile strength after 4 weeks compared to no cells and to TSG-6 silenced TSPCs. Tao *et al.* (158) delivered either normal TSPCs or TSPCs transduced with EGR1 via a fibrin sealant, and found that the presence of cells increased tissue density and Sharpey's fibers after 8 weeks; the EGR1 transduction resulted in even more Sharpey's fibers plus more type I collagen deposition.

Gumucio *et al.* (159) injected SVFCs into the muscle of the repaired tendon and observed that the cells allowed a reduction in muscle fibrosis compared to lactated Ringer's solution injection.

5. Growth factors

Therapies with growth factors are summarized in Table 5.

Uggen *et al.* (160) coated repair sutures with PDGF-BB and this increased stiffness, Young's modulus, collagen organization and fibrocartilage formation compared to normal sutures after 6 weeks. The loading with PDGF-BB of a type I collagen patch used for overlapping by Kovacevic *et al.* (122) did not change the biomechanically detrimental and histologically irrelevant outcomes of the patch alone; PDGF-BB therapy only resulted in higher cellular proliferation and angiogenesis after 5 days without any changes after 4 weeks. Hee *et al.* (136) added PDGF-BB on type I collagen disks placed at the tendon-bone interface and found that growth factor therapy with the right dosage increased load-at-failure, collagen deposition, tissue organization and quality of the tendon-bone interface after 12 weeks whereas the collagen disks alone did not elicit any improvement compared to no augmentation. PDGF-BB therapy was also used by Tokunaga *et al.* (140) who delivered it via a gelatin hydrogel sheet at the repair site and found it to increase load-at-failure, stiffness and tissue organization after 12 weeks compared to no augmentation and hydrogel alone.

There are several types of TGF-β therapies that have been studied. Kim *et al.* (161) delivered with an osmotic pump at the repair site either a "TGF-β1 treatment" (TGF-β1 and antibodies against TGF-β2 and TGF-β3) or a "TGF-β3 treatment" (TGF-β3 and antibodies against TGF-β1 and TGF-β2) or an "anti-

Table 5. Growth factor therapy

Author	Model	Tendon	Injury	Growth factors	Scenario	Analysis	Outcomes
Uggen *et al.* (160)	Sheep	IS	Chronic	PDGF-BB	Coated sutures	B,H	Increased stiffness, Young's modulus, organization and fibrocartilage compared to normal sutures after 6w.
Kovacevic *et al.* (122)	Rat	SS	Acute	PDGF-BB	Type I collagen overlap.	B,H	Similar to only scaffold. Weaker and no histological change compared to no augmentation after 4w.
Hee *et al.* (136)	Sheep	IS	Acute	PDGF-BB	Type I collagen fill.	B,H	Higher load-at-failure, organization, tendon-bone interface quality after 12w compared to no augmentation.
Tokunaga *et al.* (140)	Rat	SS	Acute	PDGF-BB	Gelatin hydrogel fill.	B,H	Higher load-at-failure, stiffness and organization after 12w compared to no augmentation and hydrogel alone.
Kim *et al.* (161)	Rat	SS	Acute	'TGF-β1', 'TGF-β3', 'AntiTGF'	Repair + osmotic pump	B,H	No histological changes after 4w. 'TGF-β1' with no effect, 'TGF-β3' decreased Young's modulus and 'antiTGF' decreased load-at-failure after 4w.
Manning *et al.* (145)	Rat	SS	Acute	TGF-β3	HBDS fill.	B,H	Higher load-at-failure, stiffness, cellularity, scar tissue and vascularity than HBDS-only and no augmentation after 4w.
Kovacevic *et al.* (146)	Rat	SS	Acute	TGF-β3	Ca-P fill.	B,H	Increased load-at-failure, stiffness and type I collagen after 4w compared to no augmentation and Ca-P alone.
Lu *et al.* (162)	Sheep	IS	Acute	FGF-2	Coated sutures	H	Similar to normal sutures after 6w.
Zhao *et al.* (129)	Rat	SS	Chronic	FGF-2	PLGA overlap.	B,H	Higher load-at-failure, stiffness and organization than no FGF-2 and no augmentation after 8w.
Peterson *et al.* (123)	Sheep	IS	Acute	FGF-2	P4HB/Type I collagen overlap.	B,H	No biomechanical changes. More bone ingrowth after 26w than no augmentation.
Ide *et al.* (142)	Rat	SS	Acute	FGF-2	Fibrin sealant fill.	B,H	Increased load-at failure and bone ingrowth after 2w compared to no FGF-2.
Ide *et al.* (102)	Rat	SS	Acute	FGF-2	ADM interpos. and fibrin sealant fill.	B,H	Increased load-at-failure, fibrocartilage deposition and tissue organization after 6 and 12w compared to no FGF-2.
Buchmann *et al.* (163)	Rat	SS	Chronic	FGF-2 or G-CSF or mixture	Repair + osmotic pump	H	Better organization with all. More type I collagen only with FGF-2 or FGF-2/G-CSF.
Buchmann *et al.* (148)	Rat	SS	Chronic	G-CSF	VPG fill.	B,H	More type I collagen after 6w than no augmentation and no G-CSF.
Dines *et al.* (164)	Rat	SS	Acute	GDF-5	Coated sutures	B,H	Higher load-at-failure, stiffness and organization than normal sutures after 3w.
Lamplot *et al.* (165)	Rat	SS	Acute	GDF-6	Adenovirus delivery	B,H	Increased tensile strength, Young's modulus and tendon healing after 2w.
Murray et *al.* (137)	Rat	SS	Acute	GDF-6	Collagen fill.	B,H	Increased load-at-failure, organization and collagen after 6w compared to no GDF-6.
Seeherman *et al.* (135)	Sheep	IS	Acute	GDF-7	HA paste or HA sponge or type I collagen or type I/III collagen fill.	B,H	Higher load-at-failure and stiffness with GDF-7/HA sponge, GDF-7/Collagen I and GDF-7/Collagen I/III compared to no augmentation, GDF-7/HA paste and collagen I/III alone after 8w. Better organization with GDF-7 after 8w.
Rodeo *et al.* (134)	Sheep	IS	Acute	Growth Factors Mixture	Type I collagen fill.	B,H	Better load-at-failure, organization, bone-cartilage formation after 12w than no mixture. Decreased stiffness after 12w.

TGF-β treatment" (antibodies against TGF-β1, TGF-β2 and TGF-β3). They found that the changes of natural TGF-β environment did not change the histological outcome after 1 and 4 weeks and on the other side both the suppression of the TGF-β isoforms and the abundance of fetal TGF-β3 reduced

biomechanical properties after 4 weeks. The loading with TGF-β3 of a HBDS gel by Manning *et al.* (145) allowed an increase in biomechanical properties, cellularity, fibrous tissue and vascularity and the biomechanical benefit provided by the HBDS gel alone after 8 weeks remained the same with the growth factor. Kovacevic *et al.* (146) loaded a Ca-P matrix placed at the healing site with TGF-β3 and observed that growth factor therapy increased load-at-failure, stiffness and type I collagen deposition after 4 weeks compared to no augmentation and Ca-P augmentation alone.

Lu *et al.* (162) used FGF-2 coated sutures for repair and found that this did not provide any histological benefit compared to normal sutures after 6 weeks. By loading a PLGA scaffold used for overlapping with FGF-2, Zhao *et al.* (129) found that growth factor therapy increased the beneficial effects of the synthetic scaffold by increasing load-at-failure, stiffness and tissue organization after 8 weeks. Peterson *et al.* (123) added a synthetic FGF-2 mimetic (several dosages) on a P4HB/type I collagen scaffold used for overlapping and observed that the growth factor did not change the biomechanical outcomes and on histology only had a small impact by increasing bone ingrowth at the tendon footprint after 26 weeks. FGF-2 was also added by Ide *et al.* (142) at the repair site via a fibrin sealant and resulted in increased load-at failure and bone ingrowth into the tendon-bone interface after 2 weeks compared to growth factor absence. The same authors also tested FGF-2 addition via the fibrin sealant in the context of ADM interposition (102) and found that growth factor therapy increased load-at-failure, fibrocartilage deposition and tissue organization after 6 and 12 weeks. Buchmann *et al.* (163) delivered at the repair site, via an osmotic pump, either FGF-2 alone or G-CSF alone or a combination of FGF-2 and G-CSF and observed that all therapies allowed an increase in tissue organization compared to no growth factors after 6 weeks but only the animals receiving FGF-2 (either FGF-2 alone or in combination with G-CSF) also had an increase in type I collagen deposition. Furthermore, FGF-2/G-CSF combination did not show an increased benefit. In another study Buchmann *et al.*(148) loaded VPG with G-CSF and found that the growth factor stimulated more type I collagen deposition after 6 weeks when loaded with the right dosage.

The coating of sutures with GDF-5 by Dines *et al.* (164) increased load-at-failure, stiffness and tissue organization compared to normal sutures after 3 weeks. Lamplot *et al.* (165) injected an adenovirus containing GDF-6 into an injured tendon and observed that this therapy resulted in increased tensile strength, Young's modulus and histological healing (no details given) after 2 weeks compared to no therapy. Therapy with GDF-6 was also used by Murray *et al.* (137) who loaded a collagen sponge placed under the defect region and found that the growth factor increased load-at-failure, tissue organization and collagen deposition after 4 and 6 weeks. Seeherman *et al.* (135) loaded different vehicles (hyaluronan paste, hyaluronan sponge, type I collagen sponge and type I/III collagen sponge) with GDF-7 and added them at the tendon-bone interface and also at the surface of the repaired tendon. Groups receiving GDF-7/Hyaluronan sponge, GDF-7/Collagen I and GDF-7/Collagen I/III had increased load-at-failure and stiffness compared to the groups receiving no augmentation, GDF-7/Hyaluronan paste and collagen I/III alone after 8 weeks, whereas upon histology assessment all the groups receiving GDF-7 treatment had better collagen alignment compared to no augmentation and collagen I/III alone after 8 weeks.

Rodeo *et al.* (134) loaded a collagen sponge placed at the tendon-bone interface with an osteoinductive bone protein extract containing BMP-2-7, TGF-β 1-3 and FGF and observed that the mixture increased load-at-failure, tissue organization, bone formation, cartilage deposition after 6 and 12 but decreased stiffness after 12 weeks compared to absence of growth factors.

6. Cells and growth factors

The combination of cell and growth factor therapies is summarized in Table 6.

Table 6. Cell and growth factor therapy

Author	Model	Tendon	Injury	Cells	Growth factors	Scenario	Analysis	Outcomes
Uggen *et al.* (166)	Rat	NS	Chronic	Fibroblasts	PDGF-BB or IGF-1 (expressed by cells)	PGA overlap.	H	Increased organization after 6w with PDGF-BB compared to no augmentation. No outcomes reported for IFG-1.
Dines *et al.* (130)	Rat	NS	Chronic	Fibroblasts	PDGF-BB or IGF-1 (expressed by cells)	PGA overlap.	B,H	Higher load-at-failure with IGF-1, not with PDGF-BB after 6w compared to only PGA. Higher organization with seeded scaffolds.
Gulotta *et al.* (155)	Rat	SS	Acute	BM-MSCs	GDF-6 (expressed by cells)	Fibrin sealant fill.	B,H	No change compared to BM-MSCs.
Chen *et al.* (150)	Rabbit	IS	Acute	PPCs	BMP-2	PEGDA fill.	B,H	Increased load-at-failure, fibrocartilage and organization after 4 and 8w compared to PEGDA alone.

The combination of cell and growth factor therapy is another option described in the literature. Uggen *et al.* (166) seeded a PGA scaffold used for overlapping with fibroblasts either transduced with PDGF-BB or IGF-1. Therapy with PDGF-BB increased tissue organization after 6 weeks compared to no augmentation at all and no outcomes for the IGF-1 group were reported. As previously seen, Dines *et al.* (130) reported that the use of a PGA scaffold to overlap the repair site did not change the outcome. They also seeded the PGA with fibroblasts transduced with either PDGF-BB or IGF-1 and found that both therapies increased tissue organization after 6 weeks compared with non-seeded scaffolds but only IGF-1 allowed at the same time an increase in load-at-failure. Gulotta *et al.* (155) added fibrin sealant containing either normal BM-MSCs or BM-MSCs transduced with GDF-6 at the repair site and found that both therapies gave the same biomechanical and histological outcomes after 5 weeks. Chen *et al.* (150) added PPCs and BMP-2 in the solution of a solidified PEGDA hydrogel applied at the tendon-bone interface of a repair and observed that cell and growth factor therapy allowed an increase in load-at-failure, fibrocartilage formation and tissue organization after 4 and 8 weeks compared to PEGDA augmentation alone.

7. PRP

Therapy with PRP is summarized in Table 7.

The use of PRP is well documented in the literature but the preparation of the therapy is variable and thus can have significant differences for overall quality of the product administered as shown by Akhundov *et al.* (167). Beck *et al.* (168) added PRP at the repair site and obtained ambivalent results with a decrease in stiffness and an increase in tissue organization after 3 weeks. The addition of PRP at the repair site was also tested by Hapa *et al.* (169) who found that it increased load-at-failure after 2 weeks and on a histological level it decreased inflammation, it allowed formation of a better tendon-bone interface and it increased collagen organization after 2 and 4 weeks compared to no PRP treatment. We previously saw that Chung *et al.* (121) showed that overlapping of the repair site with a PD patch did not change the outcomes and in the same context they added PRP either alone at the repair site or together with the PD patch overlapping and found that PRP presence increased load-at-failure and tissue organization after 8 weeks compared to no augmentation alone and PD

Table 7. PRP therapy

Author	Model	Tendon	Injury	Scenario	Analysis	Outcomes
Beck *et al.* (168)	Rat	SS	Acute	PRP added at the repair site	B,H	Decreased stiffness but increased collagen alignment after 3w compared to no PRP.
Hapa et *al.* (169)	Rat	SS	Acute	PRP added at the repair site	B,H	Increased load-at-failure, less inflammation, better tendon-bone interface and higher organization after 2 and 4w compared to no PRP.
Chung *et al.* (121)	Rabbit	SS	Chronic	PRP added at the repair site with/without PD overlap.	B,H	Increased load-at-failure and tissue organization after 8w compared to no augmentation and PD alone. No differences among PRP and PRP/PD.
Dolkart *et al.* (170)	Rat	SS	Acute	PRP added at the repair site	B,H	Increased load-at-failure, stiffness, collagen, organization and decreased inflammation after 3w compared to no PRP.
Ersen *et al.* (171)	Rat	SS	Acute	PRP added at the repair site with/without gelatin sponge fill.	B,H	Increased load-at-failure and organization after 8w compared to no repair and non-augmented repair.
Lamplot *et al.* (165)	Rat	SS	Acute	PRP added at the injury site with/without AdGDF-6	B,H	No histological benefit and higher tensile strength with PRP after 2w compared to no therapy but AdGDF-6 and PRP/AdGDF-6 were superior in tensile strength, Young's modulus and histology to PRP.
Wu *et al.* (172)	Rabbit	SS	Acute	PRP or PRP/BG added at the repair site	B,H	Increased load-at-failure and organization with PRP compared to no PRP after 6 and 12w. Further increase in load-at-failure, less inflammation, more organization and better tendon-bone interface with PRP/BG after 6 and 12w compared to no augmentation and PRP alone.
Kim *et al.* (149)	Rat	SS	Chronic	Addition of PRP or PRP/SAP KLD-12	H	No benefits with PRP compared with saline after 5w, but increased organization and vascularity with PRP/SAP.

patch alone, without observing any differences among the PRP treatment alone and the PRP/PD treatment (suggesting the absence of effect for the PD patch). Dolkart *et al.* (170) added PRP at the repair site and found it to increase load-at-failure, stiffness, collagen deposition, collagen organization and to decrease inflammation after 3 weeks compared to no PRP therapy. The delivery of PRP, either directly via injection at the repair site or via a loaded porcine gelatin sponge fixed at the repair site, was tested by Ersen *et al.* (171) who found that the two delivery methods were equivalent and both increased load-at-failure and collagen organization after 8 weeks compared to no repair and a non-augmented repair. Lamplot *et al.* (165) added PRP at the injury site with or without an adenovirus containing GDF-6 (AdGDF-6). PRP alone allowed an increase in biomechanical properties compared to no repair after 2 weeks; however AdGDF-6 therapy alone, as previously seen, was characterized by even better biomechanical properties and increased histological healing too. Addition of PRP to AdGDF-6 did not increase the benefits of the adenoviral therapy alone. Combination of PRP with other elements was also performed by Wu *et al.* (172) who added either PRP or a mixture of PRP and BG powder at the repair site. Both therapies allowed an increase in biomechanical and histological properties but PRP/BG was shown to be superior: PRP increased load-at-failure and collagen organization compared to no augmentation after 6 and 12 weeks, whereas PRP/BG allowed the same improvements and, in addition, it further increased load-at-failure and collagen organization plus it reduced inflammation and allowed formation of a better tendon-bone interface after 6 and 12 weeks. Kim *et al.* (149) injected either PRP alone or a mixture of PRP and SAP KLD-12 gel at the injury site and found that PRP alone, similar to SAP alone (previously seen), did not provide any histological benefit compared to a saline injection after 5 weeks, whereas the PRP/SAP combination correlated with better collagen arrangement and vascularity.

Discussion

Characteristics of included studies

The interest in the domain that is reviewed here seems to be increasing substantially as suggested by the increase of articles published per year (Figure 3).

As we have seen, the supraspinatus tendon of rat is the most studied tendon followed by the infraspinatus tendon of sheep, rabbits and dogs (Figure 4). Guides and reviews for the selection of animal models to study rotator cuff pathology and repair already exist (88,173–175) and to make an additional one was not the aim of the present work. Focus was on some important advantages and disadvantages of the several animal models cited. One of the first studies reporting development of a rotator cuff disease model found that only the rat and non-human primates provided sufficient similarity to humans; the similarity was assessed through study of shoulder musculature, bony anatomy, articulations and motion (176). In particular, it was observed that the supraspinatus tendon of the rat shared an important common feature with human's supraspinatus that was absent in the other models: the passage of the tendon through an enclosed arch (176). In larger animals such as rabbits, goats, sheep and dogs it is not possible to transect the supraspinatus tendon because the animals become lame (88). This is a possible reason why the infraspinatus tendon of sheep, rabbits or dogs are widely studied. More recently, another study provided evidence that subscapularis tendon of rabbit is also characterized by passage through a tunnel similar to human supraspinatus tendon but this model has not widely been studied yet (177). An advantage of large animals is that due to their size they allow implementing standard surgical repair techniques, as opposed to rats (88). Disadvantages of these larger models are the limited overhead activity of the shoulder and the increased costs and needs that limit sample size. A limitation of almost all models used is that the shoulder of these animals, as opposed to humans, is used for locomotion. Non-human primates would be the ideal model but their use is impractical because of the high costs and management needs. Derwin *et al.* (88) concluded that in the context of rotator cuff disease, the rat is the most appropriate model to study the mechanisms, the chronic scenario and biologic treatments, whereas larger animal models are better for studying mechanical repair techniques (e.g. sutures, scaffold interposition and overlapping), and the combination of biologic and mechanical repair. As previously seen, most of the injury models are acute injury models but in a clinical scenario most of the tears are chronic (16). Medical and scientific professionals must be aware that chronic injury models of the rotator cuff exist and should consider to use them whenever possible.

In vivo outcomes

The aim of this systematic review was to identify possible new therapies to apply on humans due to the evidence that was provided by animal testing. In order to do so, we tried to answer the following questions.

- ***Which materials are currently available for rotator cuff interposition and repair site overlapping in animals and what are their main advantages and disadvantages?***

The materials used for interposition and overlapping are often the same so that the two categories were put together to answer the question.

The use of SIS as an interpositional graft revealed itself to be equivalent for biomechanical properties to primary suture (95,99) or autologous tissue grafting (96,98) and may therefore be an option when these two therapeutic strategies cannot be applied (e.g. in the context of serious degeneration). For overlapping the repair site, SIS was found to increase stiffness in one study (118), whereas in another

one there was no biomechanical benefit (119). Regarding histology, SIS overlapping resulted in reduced necrosis around the sutures (98). Among the disadvantages we report that SIS interposition may result in reduced initial strength compared to primary repair of the tendon (95), reduced quality of the regenerated tissue (96,98) and persistent inflammation (96,98). Another source of concern of SIS is the induction of ectopic bone in the midsubstance of the tendon that was reported for both interposition and overlapping (98,119). The SIS graft seems to need at least 6 weeks to be completely resorbed but in the studies reporting persistent inflammation this period was longer.

Another commonly used ECM scaffold is ADM. In the context of interposition, use of ADM gave better histological and biomechanical results than no repair for a massive tear (101) and provided similar outcomes when comparing it with autologous tissue interposition (100). In a primate model ADM interposition allowed the formation of a tendon-like tissue and was well tolerated by the host with minimal inflammation (103). With ADM interposition a period of immobilization (2 weeks in the rats) seems to ameliorate the outcome (104). As for overlapping, ADM use was only reported in one study that showed that ADM overlapping was equivalent to debridement in a chronic scenario. A limitation of ADM use was the increased inflammation accompanying the healing process (100,120).

Further types of collagen-based scaffolds were also reported in the context of interposition. A type I/III scaffold allowed the formation of a tendon-like structure after 8 weeks (98) but it was accompanied by a persistent inflammation and induction of ectopic adipogenesis in the tendon. Two other studies reported the use of collagen-based scaffolds for interposition but their aim was to study the impact of cell seeding (105,106). The outcomes seen with collagen-based scaffolds (other than SIS and ADM) used to overlap the repair site were on the whole negative: a PD patch similar to SIS was found to reduce necrosis around the sutures but had difficulties to integrate with the host tissue (119). In another study the PD patch was not beneficial in either biomechanical or histological outcomes (121) so that doubts about its usefulness for overlapping persist. The use of a type I collagen patch was reported to be biomechanically detrimental and histologically not relevant (122), whereas the use of a P4HB/type 1 collagen scaffold did not provide any histological advantage (123).

Other non-collagen-based biological scaffolds were also reported for rotator cuff interposition: a scaffold with a continuous mineralized-demineralized transition derived from bovine cancellous bone allowed the formation of a nearly normal enthesis as opposed to simple transosseous repair without any side-effects reported (107). Also chitosan-based HA (108) and chitin (109) scaffolds look promising since they did not elicit a foreign-body reaction and were biomechanically superior to no repair. It must be said that in the absence of repair the controls only developed thin membranes between tendon and bone so that a comparison with other treatments is needed to assess the real benefit of these two scaffolds. As opposed to these beneficial outcomes, the use of a fibrin clot as interpositional graft revealed itself to be detrimental (110).

Synthetic scaffolds are widely studied in the context of rotator cuff repair. For rotator cuff interposition scaffolds based on PLA are promising since they have a slow degradation rate (111,112), they allow in the short term to reach similar biomechanical properties as primary sutures and in the long term similar properties seen in normal tendons (112). In two studies, the use of overlapping devices based on PLA, in particular PLLA, allowed an increase in biomechanical (125,126) and histological (126) properties. The grafting of gelatin to the PLLA patch further increased the biomechanical and histological advantages that the overlapping device provided (126). On the other side, no clear advantage was found for PLA (124) and fascia/PLLA (127) devices. Limitations of PLA-based scaffolds reside in the difficulty of these scaffolds to connect to the bone in the context of

interposition (111) and the induction of a foreign-body reaction, especially for PLA (not PLLA) (113,124). In this scenario a PLLA graft grafted with gelatin seems to be the best option.

For interposition the use of other synthetic scaffolds was reported. One group found that the use of a PLG scaffold was associated with decreased biomechanical properties compared to primary sutures in the short term (114) so that PLA-based scaffolds seem to be preferable. PGA interposition revealed itself to be superior compared to resection without repair (115) and to PLC interposition (116) since it allowed formation of a stronger and more biocompatible (in case of PLC comparison) repair. It would be interesting to have a study comparing PLA with PGA. The use of PTFE interposition was also reported (117) but no comparison with another treatment was performed so that it is difficult to make conclusions. However, it must be said that the use of this scaffold was accompanied by a foreign-body reaction.

Also, for repair site overlapping, there are other synthetic scaffolds than PLA that have been used: reinforcement of the repair with PLAGA (128) or PLGA (129) was reported to be biomechanically and histologically beneficial. The use of a PGA device, similar to PLA, did not result in a stronger and better repair (130). Also PU-based patches could be promising devices since they were reported to augment the biomechanical (131) and histological (132) properties without causing a foreign-body reaction (131,132). The use of PCL or PCL/PEO did not strengthen the repair and provided no histological advantages but PCL seems to be superior than PCL/PEO since it allowed better cellular infiltration (133).

Among the biological scaffolds SIS and ADM scaffolds are the most widely studied and their use for overlapping and interposition, in case autologous tissue is not available, is possible. These extracellular matrix derived scaffolds already have an ideal structure for cell ingrowth and matrix deposition and they were even reported to contain small amounts of growth factors that could accelerate the process of healing (178,179). Other biological scaffolds that are promising in this domain, but for which further studies are needed, are matrices with continuous mineralized-demineralized transition that support the formation of a normal enthesis and also scaffolds made of chitin/chitosan. As for synthetic scaffolds, PLA- and PGA-derived materials look promising and their effect may be improved by coating with gelatin. Further studies comparing the different synthetic materials are necessary but if biocompatibility is proven, these scaffolds may be ideal because they do not transmit diseases and are often very resistant (91).

- ***Which materials are currently available for rotator cuff repair filling in animals and what are their main advantages and disadvantages?***

For several materials used for filling it is not possible to say if, when used alone, they could improve or worsen the outcome since they were only used as controls for growth factor- and/or cell-delivery without comparison with another treatment (102,137,138,142–144,150).

The only biological materials that once added at the repair site resulted in an improvement of the repair were an HBDS gel (145), a Ca-P matrix (146), and CPS or HA ceramic powders (147). Among these materials CPS bioceramic seems to be the best option since it allowed a clear improvement of both histological and biomechanical properties (147). Among the synthetic materials, only the filling of the repair site with a biphasic scaffold with contiguous non-mineralized (PLGA) and mineralized (PLGA-HA) regions showed some advantages (151).

- ***Does adding of cells and/or growth factors improve the results?***

Addition of fibroblastic cells at the repair site, independently of the scenario, was always beneficial: fibroblasts, tenocytes and tendon-bone interface cells improved biomechanical (105,108) and histological (98,108,139) outcome. Among the histological benefits, there were increased tissue organization (108,139), more type I collagen deposition (98,108), less inflammation (98) and less ectopic tissue formation (98). The use of MDCs, although possible, was not compared with other techniques so it is difficult to make any conclusions (152).

Among stem cells, BM-MSCs are the most studied in this domain; the use of BM-MSCs was reported to be biomechanically (115) and histologically (113,115) useful. These cells were associated with an increase in tissue organization (113), type I collagen deposition (113,115) and Sharpey's fibers (115) formation. On the contrary, one study reported that these cells were not useful (141) but the same authors demonstrated that transducing BM-MSCs with either MT1-MMP (153), a matrix metalloproteinase implicated in formation of the tendon-bone interface (56), or scleraxis (154), a transcription factor involved in tendon development (52,53), allowed biomechanical and histological improvements. Another study group found that silencing TGIF1 (144), a transcription factor downregulated in chondrogenesis (180), also allowed improvements. These experiments show us that the beneficial effects provided by BM-MSCs can be augmented with gene therapy and scleraxis seems to be most effective gene to transduce although no comparisons among these different techniques were performed and are therefore needed.

The outcomes with ADSCs are fairly controversial to date because 2 studies reported beneficial effects (156,157), whereas another study showed that ADSCs reduced inflammation at the beginning but also reduce fibrocartilage deposition afterwards (138). Among the beneficial effects of ADSCs, there are increased biomechanical (157) and histological (156,157) properties such as a reduction in fatty infiltration (156). The reduction in fatty infiltration is particularly interesting, since it correlates with a reduced risk of retear (29–31) but further studies to clarify the usefulness of these particular cells are needed. In addition, it is important to mention that differences in cell processing could have a major impact on overall cellular quality and thus clinical outcome.

Addition of TSPCs at the repair site seems like a promising technique since it is associated with an increase in both biomechanical (106,143) and histological (106,158) properties. The improvement in the strength of the repair is probably in part due to the anti-inflammatory effect of TSG-6 (181–183) as demonstrated by silencing the expression of this gene (143). On histological analysis TSPCs stimulated collagen deposition (106,158), Sharpey's fibers formation (158) and decreased inflammation (106). The transduction of TSPCs with EGR1, a transcription factor involved in tendon differentiation (54,55), was found to allow further improvement in histological properties (158).

The use of SVFCs, that probably are endothelial progenitor cells and ADSCs (46,184), is interesting since automated systems that deliver these cells are available (184–186) and they have shown to allow some histological improvements (159).

Use of growth factors is extensively reported in the literature. Growth factor therapy in general correlates with tissue abundance that is beneficial especially in the case of a degenerative scenario (e.g. degenerated tendon and osteoporosis) but can be dangerous for the development or exacerbation of impingement. The addition of PDGF-BB was found to be biomechanically and histologically beneficial in 3 studies (136,140,160) that reported increased tissue organization (136,140,160) and better tendon-bone interface development (136,160). One study also reported a decrease in cross-sectional area with PDGF-BB (160). As opposed to these studies, another experiment reported no benefits with this growth factor (122).

Growth factor therapy using TGF-β isoforms showed no consensual outcomes. An environment in which TGF-β1 was overexpressed showed no benefit, whereas an environment in which all TGF-β isoforms were suppressed revealed itself to be detrimental for tendon healing (161). The administration of TGF-β3 was in one study found to be detrimental (161), whereas two other studies found improvements in biomechanical and histological properties (145,146). It is interesting to note that in one experiment, TGF-β3 therapy correlated with increased fibrous tissue deposition (145) which is unexpected since this growth factor is involved in scarless healing of the fetus (61–63).

Of the 6 studies that reported using FGF-2, only one did not report benefits (162), whereas the others support that this growth factor has the potential to increase biomechanical (102,129,142) and histological properties such as increased tissue organization (102,129,163), more fibrocartilage deposition (102), increased bone ingrowth (123,142) and higher levels of type I collagen deposition (163).

The use of G-CSF was reported to be beneficial for tissue organization (163) and type I collagen deposition (148). All the studies reporting either the use of GDF-5 (164) or GDF-6 (137,165) or GDF-7 (135) reported both histological and biomechanical advantages. The use of a mixture of growth factors containing BMP-2-7, TGF-β 1-3, and FGF showed contrasting results since it was beneficial on a histological level and increased load-at-failure but it also decreased stiffness (134).

Only 4 articles (130,150,155,166) reported the use of cell and growth factor therapy combinations and 3 of them transduced the cells with the growth factors (130,155,166). The use of PDGF-BB, IGF-1 and fibroblasts together was found to be promising but the absence of controls with cell therapy or growth factors alone did not allow to make a general conclusion (130,166). The transduction of BM-MSCs with GDF-6 did not change the outcome compared with cells alone (155). The study reporting PPCs and BMP-2 therapy without cell transduction shows promising results but also in this case there was no control with cells or growth factors alone so that it is difficult to assess the benefit of this combination (150). We can therefore say that combination of cells and growth factors has potential to be beneficial but there is not enough experimental data that allows to recommend it. Further growth factor administration routes other than cell transduction also have to be evaluated in this context.

- ***Does the use of PRP improve the results?***

Of the 8 studies reporting PRP use, 7 observed a benefit (121,165,168–172), whereas 1 observed a benefit only with a special combination with SAP gel (149). The benefits were both biomechanical (121,165,169–172) and histological (121,168–172). Among the histological improvements provided by PRP the most frequently cited was an increase in tissue organization, in particular collagen alignment (121,168–172) and other improvements were a reduction in inflammation (169,170) and the formation of a better tendon-bone interface (169). Among others, PRP therapy was found to be inferior to adenoviral GDF-6 therapy (165). In two studies it was found that combination of PRP with other elements is beneficial: these elements are bioactive glass (60% SiO_2, 36% CaO, 4% P_2O_5) powder (172) and the already cited SAP gel (149). A limitation of PRP therapy, similarly to humans (187), is the lack of standardization in the preparation of PRP. In fact, the different PRP preparations had different platelet counts, growth factors contents, storage times and activators among others.

Limitations

We wanted to develop a systematic and large overview on what has been done *in vivo* with different animal models with the aim to improve rotator cuff treatment procedures. For this reason, the aim was to be as exhaustive as possible and thus, the included articles were not based on criteria of

quality. The studies were often performed in non-standardized conditions and in some studies the medical doctors and scientists were blinded to the type of treatment, whereas in others they were not. Another limitation is the large variety of animals, tendons and techniques studied that allowed, on one side to have an overview of all the techniques, but it also made it very difficult to make comparisons.

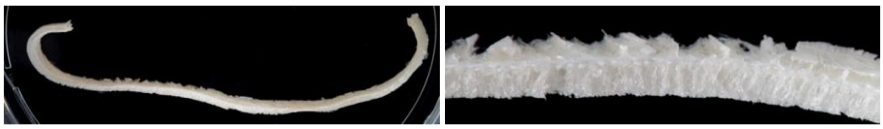
Conclusion

Among all the techniques reviewed herein, there are some that distinguish themselves for the high benefits they provide and are therefore candidates to consider for human application. In the context of interposition or augmentation SIS, ADM, PLA- and PGA-derived materials have been widely tested and showed promising results. There is however an urgent need to compare these different scaffolds between them since almost every study evaluated only one type of scaffold at a time. Other promising scaffolds are those based on chitin and those with continuous mineralized-demineralized transition. The filling of the repair site with CPS bioceramic was found to be very beneficial but again further studies would be needed. Of the different cells that have been tested fibroblasts, tenocytes, tendon-bone interface cells, BM-MSCs, ADSCs and TSPCs are the ones that gave the best outcomes and their positive effect could be further increased by transducing the cells with important transcription factors or growth factors. The growth factors that are most associated with a benefit are PDGF-BB, FGF-2 and GDFs 5-7. The combination of cell and growth factor therapy is surely a domain that has high potential to develop but further studies are needed because there is not enough experimental data to recommend any particular one over another. The application of PRP at the repair site was found to be beneficial and this effect was further increased by combination with bioactive glass powder or SAP gel.

Pilot study

Background: The aim of these experiments was to evaluate silk scaffolds as potential substitutes for injured tendons. To do so, we measured the biomechanical properties of silk scaffolds seeded or not seeded with cells and compared them to tendon tissue.

Methods: Silk scaffolds were provided by Zhejiang University and their size was 100 x 3 x 3 mm.



Silk scaffold at different magnification

The cells seeded onto the scaffolds were human fetal progenitor tenocytes (hFPTs) taken from the cell bank (UTR Biobank, Ethics protocol #62/07). More details about this cell bank are provided elsewhere (188). Passage 5 cells were seeded onto the scaffolds with the use of two different solutions, one with 31,250 cells/mL and the other with 250,000 cells/mL. In doing so we obtained scaffolds with either 5,000 or 40,000 cells/cm^2. Immediately after seeding the cells were left to adhere for 2 hours before medium was added in the Petri dish. Growth medium was changed twice a week for 30 days. After 30 days, we performed the biomechanical study. For biomechanical testing we used 5 non-seeded silk scaffolds hydrated with PBS, 6 seeded scaffolds with 5,000 cells/cm^2, and 6 seeded scaffolds with 40,000 cells/cm^2. As a comparison, we used 10 bands of horse superficial digital flexor tendon measuring 150 x 10 x 1.2 mm cut with a dermatome. Biomechanical testing was realized with a materials testing system (Instron E3000, Instron, Norwood, MA, USA) and the specimens were fixed with 2 clamps designed in collaboration with the workshop of the Institute of Mechanical Engineering of the Swiss Federal Institute of Technology in Lausanne (EPFL). We left a gap of 80 mm between the clamps and then began testing with a rate of 25 mm/min until rupture. Every 20 ms load-at-failure and deformation were recorded; afterwards we normalized the results to obtain tensile strength (load divided by cross-sectional area) and strain (deformation divided by initial length). By calculating the slope of the stress-strain curve in its linear portion we obtained the Young's modulus. Results are provided as mean value ± standard deviation.

Results: The outcomes of our analysis are reported here:

	Tendon	Silk no cells	Silk 5'000 cells	Silk 40'000 cells
Tensile strength (MPa)	9.30 ± 3.18	0.91 ± 0.18	0.79 ± 0.17	0.76 ± 0.12
Strain (%)	8 ± 1	44 ± 3	37 ± 5	41 ± 11
Young's modulus (MPa)	149.44 ± 41.20	3.01 ± 0.68	2.54 ± 0.56	2.26 ± 0.45

For all parameters studied, silk scaffolds were always clearly inferior to tendon tissue. Also the seeding of hFPTs did not reinforce the scaffolds and was even associated with a tendency towards decreasing biomechanical properties. It is possible that the process of cell seeding increased biological degradation of the scaffold but we also think that the absence of mechanical stimulation of the cells (e.g. periodical loading of the scaffold) did not stimulate the cells to produce extracellular matrix and therefore did not reinforce the structure.

Conclusion: The use of silk scaffolds as substitutes for tendons does not seem to be possible with the current formulation because their biomechanical properties are very different. Also the seeding with hFPTs did not change the outcomes but further studies are needed to see if mechanostimulation could allow the cells to increase extracellular matrix production and thus enhance overall biomechanical properties of an engineered tendon.