

New perspectives in rotator cuff tendon regeneration: review of tissue engineered therapies

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Abstract Tissue engineering may play a major role in the treatment of rotator cuff tendon lesions through replacement of an injured tendon segment. Tendons have very poor spontaneous regenerative capabilities, and despite intensive remodelling, complete regeneration is never achieved and the strength of tendon and ligaments remains as much as 30% lower than normal even months or years following an acute injury. Tendons seem to be the least complex of the connective tissues with respect to their composition and architecture and this leads to the expectation that they would be more amenable to tissue engineered approaches than other tissues. An accurate literature revision was done in order to know the state of the art of tissue engineering therapies in the field of rotator cuff regeneration. The following techniques of tissue engineering were considered: local injection of stem cells or growth factors, gene transfer, *in situ* tissue engineering and *in vitro* production of bioengineered tendons to be further transplanted in the lesion site. So far, few experimental or clinical studies have been done

on tendon tissue engineering compared to the extensive work on other tissues of orthopaedic interest, such as bone and cartilage. The existing studies are related to the following tissue engineering methodologies: gene transfer, *in situ* tissue engineering and *in vitro* production of bioengineered tendons. In our opinion the previously described literature revision showed the necessity for future studies in this area also because of recent advances in biological and bioactive scaffolds.

Keywords Rotator cuff · Tissue engineering · Scaffolds · Bioengineered tendons

Introduction

The shoulder's rotator cuff is one of the most commonly injured soft connective tissues, and when surgery is necessary to avoid patient disability, primary repairs, autografts, allografts, resorbable xenografts and synthetic biomaterials have been attempted [1–4]. However, the treatment of massive rotator cuff tears is still a major challenge in shoulder surgery [2]. More than 300,000 operations are performed each year for the reconstruction of the rotator cuff in the USA, more than 30% of rotator cuff tears may be of difficult treatment or beyond repair and failure rates after primary repair have been reported to be 13%–68% for open repairs [2, 5]. Revision rotator cuff surgery has also been shown to have less successful outcomes than primary repairs [6]. Tissue engineering may play a major role in the treatment of these injuries through replacement of an injured tendon segment [7]. Tendons seem to be the least complex of the connective tissues with respect to their composition and architecture and this leads to the expectation that they would be more amenable to tissue engineered

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approaches than other tissues [8]. Tendons are composed of highly specialised mesenchyme-derived cells (tenocytes) embedded in a three-dimensional network of extracellular matrix (ECM) consisting predominantly of collagen type I (CICP) (>95%), other types of collagens (type III and V), proteoglycans (PG), elastin and fibronectin (FBN) [9]. Tendons have very poor spontaneous regenerative capabilities [8], and despite intensive remodelling, complete regeneration is never achieved. The tissue replacing the defect remains hypercellular. The diameter of the collagen fibrils is altered, thus favouring thinner fibrils with reduction in the biomechanical strength of the tendon [10]. The strength of tendon and ligaments remains as much as 30% lower than normal, even months or years following an acute injury (<http://tendinosis.org/injury.html>).

A literature revision was done in order to know the state of the art of tissue engineering therapies in the field of rotator cuff regeneration. The following techniques of tissue engineering were considered and discussed:

- local injection of stem cells or growth factors
- gene transfer
- *in situ* tissue engineering by the use of bioactive biological membranes
- *in vitro* production of bioengineered tendons to be further transplanted in the lesion site

Results of the revision showed the necessity of further studies in this area both *in vitro* and *in vivo* in order to study idoneous scaffolds and cell types for regenerative medicine in shoulder surgery and to enhance the clinical outcomes.

Materials and methods

We searched the English language literature of the PUBMED database: keywords (all fields): “tissue engineering” and “rotator cuff”; “mesenchymal/stem cells” and “rotator cuff”; “gene transfer” and “rotator cuff”; “membranes” and “rotator cuff”. Articles were included in the review if they were related to the investigation of tissue-engineered therapies (local injection of stem cells or growth factors, gene transfer, *in situ* tissue engineering and *in vitro* production of bioengineered tendons to be further transplanted in the lesion site) on the lesions of rotator cuff tendons and if a full length paper in English language was easily available. The search included *in vitro*, animal and human studies.

Results

So far, few experimental or clinical studies have been done on tendon tissue engineering compared to the extensive work on other tissues of orthopaedic interest, such as bone

and cartilage. The existing studies are related to the following tissue engineering methodologies: gene transfer, *in situ* tissue engineering and *in vitro* production of bioengineered tendons. We obtained 15 experimental papers and, in more detail, 4 papers on gene therapy [11–14], 9 papers on *in situ* tissue engineering with the use of bioactive acellular membranes [6, 15–22] and 2 papers on *in vitro* tissue engineered tendons [23, 24].

As far as gene transfer is concerned, Dai et al. [11] performed an *in vitro* and *in vivo* study to determine if adenovirus-mediated gene transfer was feasible in healing tendons. The transfection efficacy was investigated *in vitro* with primary cultured human rotator cuff tendon cells and *in vivo* with a rat Achilles tendon healing model. Moreover, the same authors tested whether transfection efficacy and gene localisation could be enhanced using a gelatine sponge to deliver the adenoviral vector. The results demonstrated that adenovirus can be used to transfer a gene of interest to healing tendon and cultured tendon cells. A gelatine sponge implantation enhanced transfection efficiency and localisation *in vivo*. Pelinkovic et al. [12] genetically engineered muscle-derived cells. After characterisation the cells were injected into the supraspinatus tendons of nude rats. Results showed that the native tissue (tendon ECM and cells) modulated the injected cells toward a fibroblastic phenotype. In another study [13] cells isolated from rat rotator cuff tendons were transduced with the genes of either PDGF- β or IGF-I by retroviral vectors. Then they were seeded onto a polymer scaffold and also used to repair transected and then sutured rotator cuffs. Results showed that tendon fibroblasts can be tissue engineered to deliver therapeutic peptides to local environments to stimulate a repair response. The *in vivo* part of the study showed that the animals receiving tissue construct grafts containing cells expressing PDGF- β plus reconstruction with suture repaired with near complete to full restoration of the torn tendon. Dines et al. [14] used rat tendon fibroblasts that were cultured and transfected with the genes of PDGF- β or IGF-I by a retroviral vector. Then, cells were seeded on a porous polyglycolic acid scaffold. *In vitro* studies showed that the gene-modified cells had the capacity to modulate the local environment. Finally, *in vivo* tests showed the ability of the tissue engineered scaffolds to improve biomechanical characteristics of repaired tendons, in particular in the case of IGF-I transfection.

The majority of researchers working on rotator cuff tendon regeneration studied *in situ* tissue engineering both *in vitro* and *in vivo*. The present authors [15] comparatively investigated *in vitro* the behaviour of tenocytes cultured on two collageneous scaffolds: human dermal matrix and a Swine Intestinal Submucosa derived membrane (SIS) (Figs. 1 and 2). Then cell proliferation and production of ECM proteins, pro-inflammatory cytokines and growth

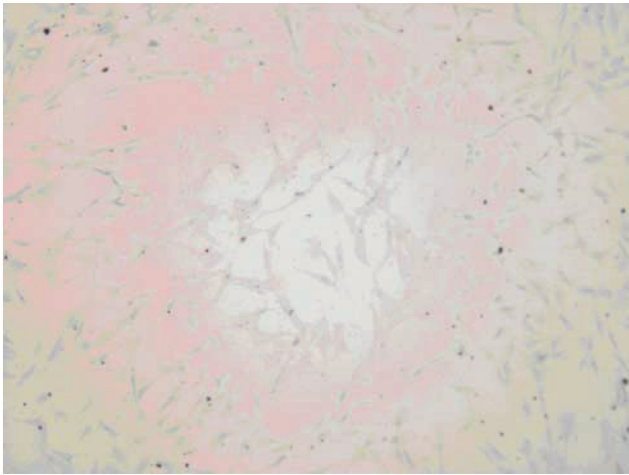


Fig. 1 Light microscopy of tenocytes isolated from biopsies of tendons. Adhesion of tenocytes to the well culture (10x)



Fig. 2 Tenocytes cultured with human dermal matrix at 7 days (light microscopy, toluidine blue, 4x)

factors were measured. Results showed that both scaffolds supported the cell growth and production of collageneous and non-collageneous proteins. However, the human dermal matrix presented more appropriate characteristics than SIS as far as cell proliferation, fibronectin and interleukin 6 increase is concerned. This cell behaviour was maintained also when tenocytes were isolated from subjects previously *in vivo* submitted to glucocorticoid administration.

The effect of anabolic steroids on human rotator cuff tendon cells was evaluated by Triantafillopoulos et al. [6] to test the possibility of enhancing the biomechanical properties of bioartificially engineered human supraspinatus tendon. The authors demonstrated that nandrolone decanoate and load acted synergistically to increase matrix remodeling and biomechanical properties of a 3-dimensional *in vitro* rotator cuff tendon model and concluded that anabolic steroids may enhance the production of bioartificial tendons and rotator cuff tendon healing *in vitro*.

In vivo, Funakoshi et al. [16] used rabbits with a 10x10 mm defect in the infraspinatus tendon. An acellular matrix made of nonwoven chitin fabric enhanced the biologic and mechanical regeneration of rotator cuff tendons as evaluated by histology, immunohistochemistry and biomechanics. The majority of experimental studies in this field were performed on SIS. Using a dog model, Dejardin et al. [17] demonstrated that SIS could be used to replace a completely resected infraspinatus tendon. They found that SIS-regenerated tendons mimicked those of tendons treated with routinely applied procedures of tendon mobilisation reimplantation at 3 and 6 months. Schlegel et al. [18] compared rotator cuff tendon repair made under tension in sheep. Differently from Dejardin, they used SIS not as tendon replacement but as augmentation in comparison with not augmented sutures. At 12 weeks they did not find significant differences between the 2 groups in histological findings and load to failure. Significant higher mean stiffness was found in augmented cases in comparison with non-augmented ones, with the SIS-augmented specimens closer to the stiffness of the normal tendon. Zalavras et al. [19] implanted SIS in large supraspinatus tendon defect of rats. They observed that SIS increased tendon neovascularisation and fibroblast growth and significantly improved ultimate force to failure in comparison with untreated defects. Perry et al. [20] for the first time investigated the role of SIS not only in acute lesions but also in chronic lesions of the supraspinatus tendon of rats. Acute repair with SIS was comparable to acute repair without SIS while in the chronic group the use of SIS significantly reduced the area of healing tendons and increased the modulus of healing tissue compared with native tendon repair. Nicholson et al. [21] studied the effect of transosseous suture repair, absorbable patch of SIS and non-absorbable patch of swine derma in ewe infraspinatus tears. They found significant levels of fibrinogen at 3 weeks in SIS-treated groups, and higher failure load in dermal-treated tendons than in suture and SIS groups at 9 weeks. At 24 weeks there were no differences among groups with diverse tissue types in SIS-treated tendons.

Most importantly, SIS therapeutic efficacy as augmentation for open repair of large and massive rotator cuff tears was also tested in 30 shoulders in a randomised controlled clinical trial by Iannotti et al. [22]. Even though a small group of patients was enrolled, results showed that SIS augmentation of the surgical repair of a chronic two-tendon rotator cuff tear did not improve the rate of healing or the clinical outcome scores. Three of 15 patients treated with SIS had a sterile inflammatory reaction.

With regards to the *in vitro* production of bioengineered tendons, Funakoshi et al. [23] developed a 3-dimensional scaffold composed of chitosan-based hyaluronan hybrid polymer fibres and tested if this novel

scaffold could enhance collagen I production with seeded fibroblasts and improved healing in rabbit models of rotator cuff defect. The study confirmed that a 3D scaffold constructed from chitosan-based hyaluronic hybrid polymer fibres enhanced the production of collagen and provided mechanical strength for the regenerated tissue of the rotator cuff *in vivo*.

Tenocyte-seeded bioscaffolds (SIS and type I/type III collagen) were implanted in rabbit rotator cuff tendon defects by Chen et al. [24] and the healing of the lesions was compared with that of those treated with bioscaffolds and autologous tendons excised during defect creation. Histological results of this comparative study suggest the great potential of autologous tenocytes cultured on collagen-based biomaterials for massive rotator cuff lesions.

Discussion

Because of its relatively avascular nature with low density of cells (20% of the tissue volume) exhibiting low mitotic activity, tendon healing takes a considerably long time and in most cases it results in the production of a mechanically inferior tissue [17]. Therefore, tendon is recognised to be a prime candidate for engineered tissue replacement [17]. However, so far, few studies have been done on rotator cuff tendon tissue engineering compared to the extensive work on bone and cartilage. The local injection of stem cells or growth factors has only been experimentally tested with good results in surgically created Achilles tendon lesions [18, 19], but no attempts have been made at rotator cuff lesion repair.

Gene therapy involves the transfer of a certain gene into a cell so that the cell translates the gene into a specific protein [14]. Literature studies show that tendon fibroblasts can be easily cultured, and transduced with genes, and therefore that viral mediated transfer in rotator cuff tendon cells and tendon healing *in vivo* is feasible [11, 13, 14]. The use of muscle-derived progenitor cells and *ex vivo* gene therapy could represent an interesting application in the treatment of rotator cuff lesions because of the demonstrated capability of these cells to differentiate into fibroblasts when implanted *in vivo* [12]. For treatment of rotator cuff tears, the major concern of using gene therapy is safety, and a large number of basic science and preclinical studies still need to be performed before the clinical application in this field.

Another “simpler” approach in rotator cuff tendon regeneration is to design a reliably bioactive scaffold capable of increasing angiogenesis and recruiting and stimulating the patient’s own cells [25]. Many biological membranes simulating ECM are now available for rota-

tor cuff tendon regeneration but nowadays their clinical use is approved for tendon reinforcement and not replacement [26, 27]. The advantage of this approach versus *in vitro* tissue engineering is that “one step” surgery is performed in the absence of the need of cell manipulation. However, biological membrane immunogenicity and adverse tissue reaction is a critical aspect mainly depending on the decellularisation technique that, ideally, has to eliminate cell remnants but preserve, as much as possible, ECM chemistry, biology and mechanical properties [26, 27]. The most investigated acellular scaffold for rotator cuff tendon regeneration is SIS. This bioactive membrane after implantation promotes a reconstructive healing response through angiogenesis and migration and differentiation of host cells, but neither Iannotti et al. nor Sclamberg et al. found outcome improvements in patients after SIS reinforcement of large and massive rotator cuff tears [5, 22]. A non-specifically inflammatory reaction was reported by Malcarney et al. in 4 of 25 patients submitted to SIS implantation for rotator cuff tears at a mean of 13 days after surgery [28]. Moreover, Zheng et al. [29] assessed safety and efficacy of the SIS membrane and found that it is not an acellular membrane but contains porcine DNA and elicits an inflammatory reaction when implanted heterotopically and orthotopically in laboratory animals.

Briefly, the surgically implanted scaffolds themselves have to facilitate cell or growth factor recruitment and tissue regeneration in the *in vivo* environment [30] but the importance of accurate preclinical studies to evaluate biomaterials in depth for successful bioscaffold design and to avoid patient complications is to be highlighted and, also in our opinion, deserves more attention from researchers and clinicians.

As far as the *in vitro* production of bioengineered tendons is concerned, the feasibility of rotator cuff regeneration using current tissue engineering techniques with 3D scaffolds has also been demonstrated [23, 24]. The necessity of long times, the accurate selection of cell source (differentiated *vs.* mesenchymal undifferentiated cells) in the absence of invasive tissue harvesting procedures for autologous cell yield together with strict legislative aspects due to the extensive cell manipulation are to be taken into account in comparison with “one step” tissue engineering. Adjunctive methods for improving bioartificial tendon properties could be represented by appropriate drug administration and as suggested by some authors, further *in vitro* and *in vivo* studies on this topic may be useful [6].

In conclusion, few *in vitro* studies have been performed by comparing different scaffolds for rotator cuff tissue engineering. *In vivo* different animal models such

as mice, rats, rabbits, sheep and dogs were used. Recent papers have described in detail the advantages and disadvantages of both rabbit and sheep models, thus contributing to the refinement of *in vivo* research [31, 32]. However the frequently used acute tear model does not replicate the typical clinical scenario of human beings, which is a chronic tendon tear [21]. Finally, many *in vivo* studies compared scaffold-treated lesions with untreated ones so that it is really difficult to understand the actual advantages of *in situ* tissue engineering vs. traditional surgical techniques of tendon repair.

These reasons and the small number of studies in the previously described literature revision show the necessity for future studies in this area. In particular, there is the lack of comparative and reliable preclinical studies and of prospective randomised clinical trials.

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