

Tendon Healing: Biological Principles And Augmentation with Autogenous Mesenchymal Stem Cells, Current Concepts

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shown promising results in augmenting the healing process of either ruptured or degenerative tendons. More human studies should focus on what seems a currently narrow but genuinely promising field regarding tendon augmentation procedures.

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Key words: Tendon; Healing; Mesenchymal; Stem cell

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ABSTRACT

OBJECTIVE: The aim of this study is to produce a critical analysis about the properties of human tendons and review any progress that has been developed in tendon augmentation with mesenchymal stem cells.

MATERIALS/SUBJECTS AND METHODS: Currently published literature, written in English and distributed via Medline, Embase, Google Scholar and Cochrane Library was studied. References of retrieved articles were also analysed for possible inclusion.

RESULTS: A concise description of tendon characteristics and process of tendon healing process is provided. Stem cells varieties are reported, along with harvesting possibilities, most promising being adipose and mesenchymal stem cells. Current methods of stem cell application are via scaffold creation, cell-seeded materials to the degenerative tendon and cell injection. So far only stem cell injection has been involved in human studies and relevant applications in equinus tendinopathy have been also described. Several authors have also investigated stimulation of stem cells by mechanical means or growth factors; almost all studies have demonstrated positive results.

CONCLUSION: In any way, the application of stem cells has

INTRODUCTION

Tendons are tough fibrous structures that connect muscles to bones and withstand exceptionally high tensile loads^[1]. By transmitting muscular forces to bones, the tendons largely contribute to joint and body movements. However, continuously bearing loads may lead to tendon injuries and tears^[2]. Tendons generally heal slowly due to poor blood supply. The strength of the created scar tissue is biomechanically weaker than the original tendon and therefore a significant risk of re-rupture exists^[3]. Functional restoration of the injured tendon still presents a challenge and it is mainly affected from both intrinsic and extrinsic factors^[4].

Tendon anatomy

The synthesis of a tendon includes water, collagen (mostly Type I, 98%), elastin, proteoglycans such as decorin and aggrecan, and cells such as tenoblasts and tenocytes. The structure is multi-unit hierarchical and contains fibrils, fibre bundles, fascicles and tendon unit^[5]. Microscopically, tendons have a crimped waveform appearance that disappears when stretched and reappears when unloaded as a result of the cross-linking of proteoglycans. This crimp pattern contributes to a tendon's task, as on a repaired tissue it has

been found smaller and with decreased functionality^[5]. Along with the crimp pattern, cells sense any mechanical forces and convert them into biochemical signals that lead to adaptive physiological or pathological changes. Through these alterations a tendon adapts and changes its structure, composition and ultimately mechanical properties^[5].

Tendons can be categorised as intrasynovial if they are surrounded by a sheath or extrasynovial if they are not. Both tendon types are similar in terms of composition and architecture but different regarding their nutrition and mechanical properties. An intrasynovial tendon, such as the flexor digitorum profundus, receives most of its nutrition by diffusion of elements from the synovial sheath and it has a minimal direct blood supply. Extrasynovial tendons, like Achilles tendon, receive nutrition directly from arteries deriving from the periosteum, musculotendinous junction, surrounding soft tissues and paratenon (if it is apparent)^[6-7]. They have greater tensile strength but weaker compressive properties compared to intrasynovial ones^[8].

Tendons are tissues with viscoelastic behaviour that can be stretched or lengthened up to a certain elastic limit. Specifically, when a strain of up to 4% is applied, tendons act in an elastic manner and reform to its original shape and performance after release of the strain. Strains in excess of 4% cause microscopic and macroscopic failure of the tissue, known as plastic deformation^[9]. Tendons contain also mechanoreceptors that can detect changes in tension, speed, acceleration, direction of movement, and position of the joints. They are activated by stretch stimuli and adjust muscle contracture in order to maintain the position and stability of the joints^[4].

Tendons have a relatively low metabolic rate. They consume small amounts of oxygen, as they obtain most of their energy from anaerobic pathways such as glycolysis and pentose phosphate cycle. The low metabolic rate of tendon tissue contributes to its resistance to withstand loads and remains in tension for long periods of time^[10]. On the other hand, the low metabolic rate results in a slow adaptive response of tendon to changes in loading, and a slow rate of recovery and healing after injury.

Pathophysiology of Tendon Disorders

Tendon injuries can be acute or chronic. Physical load, local environment, occupation and training are considered the main extrinsic factors affecting tendon performance in trauma. In addition, age, gender, systemic diseases like rheumatoid arthritis, medications like fluoroquinolones or statins, and genetic predisposition contribute the intrinsic factors leading to tendon tear^[4,11-19].

Apart from acute ruptures, chronic tendon disorders may also be encountered. Tendinopathy, tendinosis and tendinitis are common tendon disorders that may cause significant functional impairment and even tear^[3,20]. Tendinitis describes a condition where active inflammation of the tendon is implied, whereas tendinosis and tendinopathy are more general terms that refer to a pathological state of a tendon^[3,20]. Tendinosis is referred to a degenerative tendon without accompanying inflammation. Tendinopathy includes an inflammatory reaction of tendon tissue as a result of tear or vascular impairment^[15]. Tenosynovitis is a term used when inflammatory changes in synovial sheaths are apparent^[1]. The exact mechanisms and the inflammation pathways that lead to tear of tendon regeneration behind these conditions are still, to a large extent, unknown and therefore the outcome evaluation cannot be easily defined.

Recent studies examining the macroscopic and microscopic changes of tendon disorders have demonstrated that little or no

inflammation is actually present in tendons exposed to overuse^[21-23]. Histopathologic changes associated with tendinopathy include degeneration and disorganization of collagen fibers, increased cellularity but minimal inflammation response^[21,23]. Tendon thickening and loss of mechanical properties were also found^[23]. Molecular alterations have been also detected in overuse and acute injuries. These include release of matrix metalloproteinases (MMPs), increased tendon cell apoptosis, increased mucin content and chondroid metaplasia, and expression of protective factors such as insulin-like growth factor 1 (IGF-1) and nitric oxide synthetase (NOS)^[24-31]. Although the majority of these changes are pathologic and result in tendon degeneration, others appear beneficial. It seems that the tendinopathy is the result of imbalance between the protective/regenerative changes and the pathologic responses deriving from tendon overuse^[32].

Biology and biomechanics of tendon healing

Tendon healing occurs in three major phases that are not clearly distinguishable^[10]. The first stage is the inflammatory stage that begins after injury and continues for hours to a few days. Afterwards, the remodelling stage comes with collagen type III synthesis. The final stage is remodelling, which lasts approximately 6 weeks and divided into a consolidation and a maturation phase. During that time, the healing tissue is resized and reshaped. In consolidation phase, collagen type I synthesis is initiated. In maturation phase, the fibrous tissue transforms slowly (within several months) into scar tissue^[3-4,10].

Tendon healing studies have mainly been performed on either transected animal tendons or ruptured human tendons. However, their relevance to human tendinopathy with its associated healing failure response remains unclear^[4]. It is generally accepted that tendons heal by formation of scar tissue due to proliferation of the peritendinous connective tissues. Collagen is the major component of the newly created tissue but the fibres are randomly arranged in all directions. There is also a difference regarding the types of collagen being produced, especially in tendinopathy^[33-35]. The biochemical properties of scarred tendon tissue are not the same compared to normal uninjured tendons^[3,34-35]. Scarred tendons have lower capability of withstanding and transferring forces, and therefore the risk of re-rupture is greater.

Treatment Methods for Enhancing Tendon Healing

In order to restore the injured tendon properties and optimize tendon healing, several studies and experiments describing different biological treatment modalities have been published. Biological augmentation of tendon healing has been investigated by using local delivery of growth factors^[36-37], stem-cell^[38] and tendon-derived cell therapy^[39], gene-therapeutic approaches based on vehicles encoding selected factors and combinations of the above^[4]. Although many of these studies have produced optimistic results, only few have been applied to animals and only one to humans, due to lack of robust clinical studies. The equine industry shows at present an increased interest for such methods of enhancing the traditional approaches of Achilles tendon tears and disorders in horses^[40].

Regarding stem-cells studies, various tissue engineering approaches using mesenchymal stem cells (MSC) have been proposed to facilitate *in vivo* regeneration of damaged tissue or to reconstitute tendon tissue *in vitro*^[39,41]. This is achieved after differentiation of MSCs to biomechanically superior tendinous or ligamentous tissues after interaction with the local environment^[4], which holds the promise of restoring a tendon to its pre-pathological function.

STEM CELLS

1. Nature and origin of mesenchymal stem cells

Mesenchymal stem cells (MSCs) have been the focus of scientific interest for many years due to their healing promoting properties^[42-43]. Stem cells can differentiate into a variety of cell types including osteoblasts, chondrocytes, adipocytes, myoblasts and fibroblasts^[44]. A stem cell by definition is a cell type which, in the adult organism, can continue to proliferate in spite of the physiologic or artificial removal of cells from the population^[43]. The differentiation of MSCs into different phenotypes is strongly associated with the interaction between intrinsic genomic potential and extrinsic local signalling. Both mechanisms are combined at each lineage step in order to complete the developmental pathway of the emerging tissue^[43].

Stem cells may derive from different sources by using different harvesting techniques. Specifically, they can be harvested from bone marrow, periosteum, muscle connective tissue and adipose tissue^[45,46]. Although they can be found in almost all human organs^[47], bone marrow remains the most popular source of MSCs^[44]. Adult stem cells from bone marrow are usually obtained by aspiration from the anterior superior iliac crest^[44]. The age of the donor, the aspiration site, and the systemic disease state may affect the number of harvested MSCs^[48]. Approximately, there is one mesenchymal stem cell for every 100,000 nucleated marrow cells in a young healthy donor^[49].

Adipose-derived mesenchymal cells (ASCs) offer also great promise for cell-based therapies^[50]. Due to ease of harvest and abundance, ASCs have gained increasing popularity in many clinical applications. ASCs are harvested from adipose tissue derived from subcutaneous surgery. Stem cells are then isolated using enzymatic digestion, filtration and centrifugation of the stromal vascular fraction (SVF) that contains the stem cells along with non-adherent cells such as red blood cells^[51-53].

Stem cells can be also found in tendons along with differentiated tenocytes. Zhang and Wang in a recent study^[39] analysed the properties of tenocytes and tendon stem/progenitor cells (TSCs). TSCs exhibited distinct properties compared to tenocytes, including differences in cell marker expression, proliferative and differentiation potential, and cell morphology in culture. However, the option of using TSCs to more effectively repair or regenerate injured tendons should be further evaluated and seems less practical than the use of MSCs^[54].

2. MsCs: Applications in tendon disorders

2.1. Scaffolds

Mesenchymal stem cells and precursor cells are ideal candidates for tendon and ligament tissue engineering. MSCs have been mostly applied for creation of tendon grafts or augmentation of tendon grafts incorporation^[32,54-57]. Scaffolds are necessary for accommodating cell growth and tissue genesis and providing structural and mechanical support. Lack of inflammatory or immune reaction as well as cellular integration, proliferation, and differentiation into the target tissue are key concerns for the selection of the optimum scaffold^[58]. Moreover, the anabolic rate of the implanted tissue should exceed the degradation rate and the catabolic products should be eliminated through natural metabolic pathways^[59].

A scaffold for tendon engineering should have similar characteristics to tendons, native extracellular matrix, and potential of cell seeding^[60]. In Omae *et al.*'s animal study^[60], decellularized multilayer tendon slices were seeded with bone marrow stromal cells (BMSCs). The seeded cells and the collagen fibers of the

tendon slices were aligned after histology control. qRT-PCR analysis revealed greater tenomodulin and MMP13 expression and lower collagen type I expression in the composite than in the BMSCs before seeding. The results of the study suggested that BMSCs could express a tendon phenotype in this environment^[60].

Natural scaffolds include the use of collagen, hyaluronic acids, calcium alginate, and chitosan. Majima *et al.*^[61] noticed that a hybrid chitosan-based hyaluronic acid scaffold had better mechanical properties and exhibited reduced toxicity and inflammation after implantation. Sawaguchi *et al.*^[62] investigated the effect of various cyclic mechanical stresses on cell proliferation and extracellular matrix production. A biodegradable three-dimensional scaffold made from chitosan and hyaluronan was used for ligament and tendon tissue engineering. In vitro acceleration of tissue regeneration in ligament and tendon tissue engineering was found.

Synthetic scaffolds are advantageous for tissue-engineered constructs due to increased control of material properties. The most commonly used synthetic scaffolds have been made of poly-lactic acid (PLA)^[11,18-20] and poly lactic-coglycolic acid (PLGA). Ouyang *et al.*^[63] examined the abilities of bone marrow stromal cell-seeded knitted PLGA fiber scaffold in repairing the Achilles tendon. They found that the implanted allogeneic MSCs could survive for as long as 8 weeks at the rabbit lesion area. Furthermore, they could differentiate into spindle-shaped cells 5 weeks after implantation within the rabbit tendon wound site. In another study, the same authors studied the results of using knitted PLGA scaffold loaded with bone marrow stromal cells for the repair and regeneration of rabbit Achilles tendons^[64]. The knitted PLGA biodegradable scaffold loaded with allogeneic bone marrow stromal cells managed to regenerate and repair the gap defect of Achilles tendon to a statistically significant degree when compared to the control group.

2.2. Cell-seeded materials

Delivering of mesenchymal stem cell-seeded implants to a tendon gap may improve repair biomechanics. In a previous study, cultured, autologous, marrow-derived mesenchymal stem cells were suspended in a collagen gel delivery vehicle^[65]. Mesenchymal stem cells were used in a collagen matrix for Achilles tendon repair. The cell-gel composite was subsequently contracted onto a pretensioned suture. The resulting tissue prosthesis was then implanted into a 1-cm-long gap defect in the rabbit Achilles tendon. The results indicated that introduction of mesenchymal stem cell-contracted, organized collagen implants to large tendon defects can significantly improve the biomechanics, structure, and probably the function of the tendon after injury. Another study showed that pluripotential embryonic cells could be seeded onto sutures, adhere, and survive in culture. The coating of sutures with poly-L-lysine and fibronectin offered significant improvement in retention of viable cells^[66].

2.3. Cell injection-implantation

Injection and implantation of MSCs in tendon defects has been also studied in experimental animal studies^[67-70] and one human study^[71]. Awad *et al.*^[67] implanted MSCs in surgically induced rabbit patellar tendon defect. Delivering a large number of mesenchymal stem cells to the wound site resulted in a significant improvement of the biomechanical properties of the tendon. However, the implantation produced no visible improvement in tendon's microstructure.

MSCs were found also to restore the native structure of the tendon to bone junction healing in a study where hallucis longus tendons were translated into 2.5-mm-diameter calcaneal bone tunnels^[72]. Moreover, Soon *et al.* examined the effect of coating allografts

with MSCs on the quality and rate of osteointegration at the allograft tendon and bone interface. The authors noticed that MSCs significantly improved the biologic properties of soft tissue allograft healing^[56].

The use of MSCs has been also expanded in the equine thoroughbred industry to treat flexor digitorum superficialis (FDS) tendinopathy. Pacini *et al*^[73] treated 11 horses suffering from FDS tendinopathy by using a targeted intralesional injection of MSCs. Significant clinical recovery was achieved in 9 of 11 horses. In a controlled trial Smith *et al*^[74] demonstrated that intratendinous injection of 10 million autologous MSCs led to statistically significant improvement in tendon cross-sectional area, cellularity, crimp pattern and DNA content compared to controls. A post-mortem study including 17 tendons on 12 horses showed that MSCs resulted in tendon healing with minimal inflammatory cells and crimped organized collagen fibers. Furthermore, no formation of ectopic or neoplastic tissue was noticed^[40].

Despite promising findings from animal studies, only one cohort study exists so far that has tried injecting mesenchymal stem cells along with a surgical repair of a rotator cuff tear. Ellera Gomes *et al*^[71] followed a cohort of 14 patients with complete rotator cuff tears who were treated with surgical fixation and injection of autologous bone marrow mononuclear cells (BMMC). At 12 months, an increase of UCLA score from 12 +/- 3.0 to 31 +/- 3.2 was reported and at the end of follow-up only one patient deteriorated in terms of pain and strength.

2.4. Mechanical stimulation-Growth factors

Mechanical loading of MSCs on specific scaffolds may improve the quality of the new tissue^[75]. Garvin *et al*^[76] found that tendon cells fabricated in a mechanically loaded, linear collagen gel construct assume a phenotype that is similar to that of a native tendon in terms of appearance and expression and are stronger than non-exercised counterparts yet far weaker than native adult tendons^[76]. Moreover, Butler *et al*^[41] noticed that mechanical stimulation significantly enhanced tendon repair biomechanics.

Growth factors have been also suggested to play a significant role in MSCs maintenance and differentiation. At low doses, fibroblast growth factor (FGF)-2 may stimulate the proliferation of BMSCs and the upregulation of key extracellular components of tendon and ligament tissue^[77]. Moreau *et al*^[78] noticed that transforming growth factor beta (TGF β) improves cell ingrowth and collagen deposition. They also found that when using a sequential growth factor approach (growth factor basic FGF (bFGF) followed by TGF β) to in vitro human BMSCs, a significant in vitro ligament development should be anticipated.

SUMMARY

MSCs have shown so far a great potential for enhancing repair of the tendon tissue. When a tendon gap is too large to be filled otherwise, then a MSC enhanced scaffold may offer a great choice for re-establishing function of the related muscle. Cell-seeded materials, as MSC improved sutures could reduce scar tissue formation as well as rupture recurrence. A tendon suffering from a chronic tendon disorder could benefit from cell injection/implantation that can promote physiological healing and restoration of the biomechanical properties. Mechanical stimulation of MSC structure or promotion of MSC function via growth factors injection may further improve the properties of the pathological tendon.

However several aspects of tendon repair with MSCs need to be

further investigated. Information regarding the optimum type of MSCs and the proper number of cells that need to be implanted is required. Also, there is a need to clarify what is the best method of delivering MSCs into a tendon tissue. Finally, due to lack of clinical evidence, human trials examining the efficacy of MSCs in tendon regeneration and repair are necessary in order to materialise and make applicable these options of tendon repair to a human patient.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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