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Regenerative Medicine for Tendon Regeneration and Repair: The Role of Bioscaffolds and Mechanical Loading

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1. Introduction

Tendons are soft connective tissues, which connect muscle to bone forming a musculo-tendinous unit, whose primary function is to transmit tensile loads generated by muscles to move and enhance joints stability.
Adult tendons have relatively low oxygen and nutrient requirements, low cell density, and poor regenerative capacity.
The biomechanical properties of tendons are mainly attributed to the high degree of organization of the tendon extracellular matrix, primarily composed of collagen type I, arranged in triple-helical molecules bundles that have different dimensions and which are aligned in a parallel manner in a proteoglycan matrix (Fig. 1).

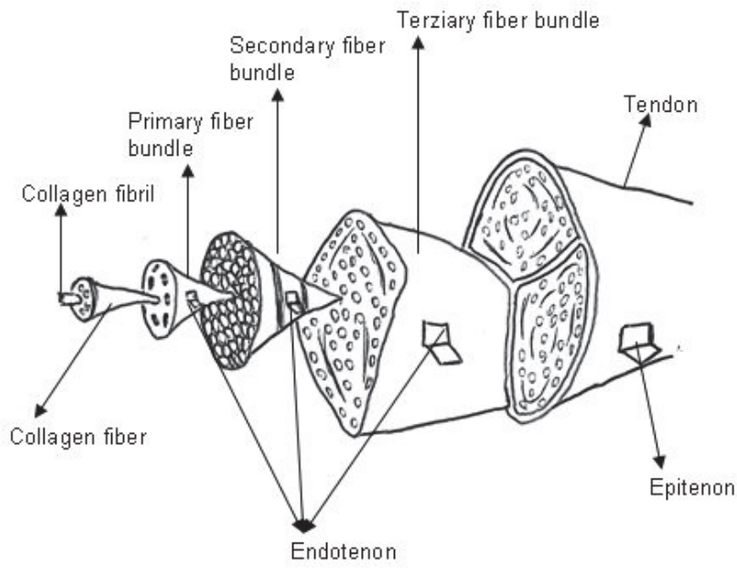

Fig. 1. Hierarchy of tendon structure.

Tendon injuries produce considerable morbidity and affect the quality of life, the disability that they cause may last for several months despite what is considered appropriate

management. The basic cell biology of tendons is still not fully understood, and the management of tendon injury poses a considerable challenge for clinicians.

Clinical approaches to tendons rupture often involve surgical repair, which frequently implies working with degenerative, frayed tendon tissue, unable to sustain the rigors of normal activities, and may fail again.

After an injury, the healing process in tendons results in the formation of a fibrotic scar and it is accompanied by an increased risk of further damage. (Longo UG et al. 2010)

The structural, organizational, and mechanical properties of this healed tissue are insufficient as tendons possess a limited capacity to regenerate.(Wong JK et al. 2009) (Woo SL et al.2006)

Adhesion formation after intrasynovial tendon injury poses a major clinical problem. Disruption of the synovial sheath at the time of the injury or surgery allows granulation tissue and fibroblasts from the surrounding tissue to invade the repair site. Exogenous cells predominate over endogenous tenocytes, allowing the surrounding tissue to attach to the repair site, resulting in adhesion formation. (Wong JK et al. 2009) (Woo SL et al.2006)

Despite remodeling, the biochemical and mechanical properties of healed tendon tissue never match those of intact tendon. It is well demonstrated that mechanical loading plays a central role in tenocyte proliferation and differentiation, and that the absence of mechanical stimuli leads to a leak of cellular phenotype. (Wang JH 2006) (Woo SL et al.2006)

While certain tendons can be repaired by suturing the injured tissue back together, some heal poorly in response to this type of surgery, necessitating the use of grafts.(Kim CW & Pedowitz RA 2003)

Unfortunately, finding suitable graft material can be problematic and biological grafts have several drawbacks. Autografts from the patient are only available in limited amounts, they can induce donor site morbidity, while allografts from cadavers may cause a harmful response from the immune system besides also being limited in supply. In both cases, the graft often does not match the strength of the undamaged tissue.(Goulet F et al. 2000) For this reason, obtaining tendinous tissue through tissue engineering approaches becomes a clinical necessity.

Tissue engineering is a multidisciplinary field that involves the application of the principles and methods of engineering and life sciences towards i) the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and ii) the development of biological substitutes that restore, maintain or improve tissue function.

The goal of tissue engineering is to surpass the limitations of conventional treatments based on organ transplantation and biomaterial implantation. It has the potential to produce a supply of immunologically tolerant, 'artificial' organs and tissue substitutes that can grow within the patient. This should lead to a permanent solution to the damage caused to the organ or tissue without the need for supplementary therapies, thus making it a cost-effective, long-term treatment.

The tissue-engineering approach involves the combination of cells, a support biomaterial construct, and micro-environmental factors to induce differentiation signals into surgically transplantable formats and promote tissue repair, functional restoration, or both.

The earliest clinical application of human cells in tissue engineering, started around 1980, was for skin tissue using fibroblasts, and keratinocytes, on a scaffold. During the last 30 years, many innovative approaches have been proposed to reconstruct different tissues: skin, bone, and cartilage. The field of tendon tissue engineering is relatively unexplored due to the difficulty in *in vitro* preservation of tenocyte phenotype, and only recently has

mechanobiology allowed a better understanding of the fundamental role of *in vitro* mechanical stimuli in maintaining the phenotype of tendinous tissues. This chapter analyzes the techniques used so far for the *in vitro* regeneration of tendinous tissues.

2. Scaffolds requirements for tendon tissue engineering

The scaffold should encourage cellular recruitment and tissue ingrowth. Early in the repair process, the scaffold should maintain its mechanical and architectural properties to protect cells and the new, growing tissue from strong forces and early inflammatory events. Subsequently, the scaffold should be gradually reabsorbed allowing a controlled exposure of the regenerating tissue to the local cellular, biochemical and mechanical environment. This will allow the tissue to develop more naturally and function more efficiently.
In order to avoid stress shielding, the scaffold should ideally degrade at the same rate that the new tissue is created. In order to ensure final clinical use, neither the scaffold nor its degradation products should be harmful to the surrounding tissue and they should not result in unresolved inflammation or other deleterious biological responses.
The tendon tissue engineering aims to repair tendon lesions in situ by integrating engineered, living substitutes with their native counterparts in vivo (Fig. 2). For this purpose, scaffolding materials are needed, and these ideally should fulfill the following requirements (Liu Y et al. 2008):

- Biodegradability with adjustable degradation rate.
- Biocompatibility before, during and after degradation.
- Superior mechanical properties and maintenance of mechanical strength during the tissue regeneration process.
- Bio-functionality: the ability to support cell proliferation and differentiation, ECM secretion, and tissue formation.
- Processability: the ability to be processed to form desired constructs of complicated structures and shapes

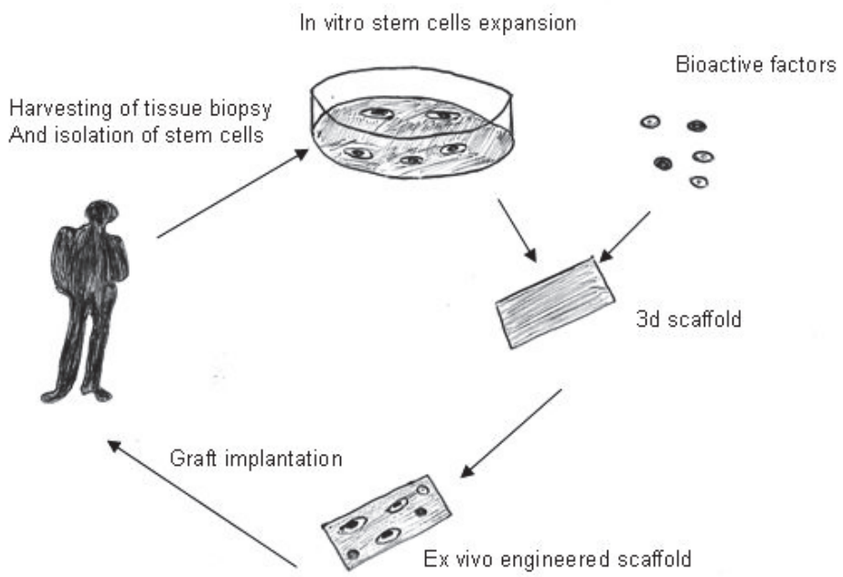

Fig. 2. Overview of tissue engineering approaches employing cell-polymer constrcuts.

In response to these varied criteria, a number of scaffold materials have been examined as scaffolds: porcine small intestine submucosa, (Musahl V at al. 2004) (Rodeo SA et al. 2004) silk fibers,(Altman et GH al. 2002) (Chen J at al. 2003) (Altman GH et al. 2003) semitendinosus tendon (Martinek V 2002), fibronectin/fibrinogen fibres. (Ahmed Z et al. 2000)
In addition to these, historically, three major categories of scaffolding materials have been employed. These are polyesters, polysaccharides, and collagen derivatives.
However, approaches to mimic the native extracellular matrix of tendon have limitetd to their inappropiate mechanical strength or the lack of cell adhesion sites. The use of an acellular graft may prevent the initial cell necrosis observed when autografts and allografts are used wich leads to the deterioration of mechanical strength following implantation.
Natural tissue scaffolds have the advantage of preserved ECM proteins important for cell attachment and the desired mechanical properties.
Natural scaffolds are composed of extracellular matrix proteins that are conserved among different species and wich can act as scaffolds for cell attachment, migration and proliferation.
Natural scaffolds have been decellularized in order to reduce their immunogenicity, a major hurdle to overcome in acellular scaffolds is their capacity for recellularization and regeneration with cellular components in vitro or in vivo, in order to achieve optimal biological and biochemical functions.(Gilbert TW 2006)

2.1 Collagen scaffolds

Collagen derivatives have been intensively investigated for use in tendon tissue engineering applications. Tendon extracellular matrix are mainly composed of type I collagen, so scaffolds based on collagen derivatives are highly biocompatible, then collagen derivatives also exhibit superior bio-functionality: they better support cell adhesion and cell proliferation.
Cells cultured in collagen gels produce extracellular matrix and align longitudinally with the long axis of the tissue equivalent, thereby mimicking cell alignment in ligaments *in vivo*.(Goulet F et al. 2000) (Huang D 1993)
Fibroblasts seeded in collagen gels change their shape and orientation over time (Huang D 1993) (Bell E at al. 1979) (Klebe RJ et al.1989) (Nishiyama T et al. 1993) and these organizational changes have been correlated with cell proliferation, protein synthesis, and matrix morphogenesis. (Ben-Ze'ev A et al. 1980) (Harris AK et al. 1981) (Maciera-Coelho A 1971) Fibroblast-seeded collagen scaffolds have been investigated with regard to their ability to accommodate cell attachment, proliferation, and differentiation. (Goulet F et al. 2000), (Huang D 1993) (Bellincampi LD et al. 1998) (Dunn MG et al. 1995)
Collagen gel has been reported to augment the quality of tendon repair, but collagen gel does not possess sufficient mechanical strength, it is often accompanied by a high-strength component. For instance, Awad et al. (Awad HA et al. 2003) studied collagen gels in combination with a polyglyconate suture for patellar tendon repair. The biomechanical properties of the resulting tendon tissues were significantly better than those of naturally healed tendons, yet still much inferior to those of uninjured tendons.
Compared with collagen gel, collagen sponges exhibit greater mechanical competence. Given that collagen gels exhibit superior cell-seeding efficiency, a combination of collagen gels with collagen fibres or sponges represents a promising strategy. Juncosa-Melvin et al. (Juncosa-Melvin N et al. 2006) showed that gel–collagen sponge constructs could greatly enhance functional tendogenesis.

Another study, (Gentleman E et al. 2006) provided further evidence that a combination of collagen gels and sponges could bolster development of tendon-like tissue.
Despite its superior bio-functionality and biocompatibility, remaining several limitations to collagen. First, its processability is limited, the degree to which collagen scaffolds can be characterized is restricted. Then, the mechanical strength of collagen scaffolds is much lower than other materials such as the polyesters.

2.2 Polyesters scaffolds

A vast majority of biodegradable polymers for tendon tissue engineering applications are polyesters, such as polyglycolic acid (PGA), polylactic acid (PLA) and their copolymer polylactic-coglycolic acid (PLGA).These polymers are attractive, because their degradation products, glycolic acid and lactic acid, are natural metabolites that are normally present in the human body. Moreover, their good mechanical properties and their processability increase their appeal.
PLGA scaffolds have been reported to improve tendon regeneration considerably. Ouyang et al. (Ouyang, H.W. et al. 2003) found that knitted PLGA scaffolds augmented the tendon healing, both histologically and mechanically. These scaffolds facilitated production of collagen type I and type III fibrils and contributed to the improved mechanical properties.
PGA was also reported as a scaffolding material, Cao et al. (Cao Y.et al. 2002) developed a PGA scaffold that could successfully restore the mechanical capacity of tendons in a hen model. Moreover, Wei et al. (Wei X. et al. 2005) found that woven PGA scaffolds were particularly suitable for tendon tissue engineering because they surpassed the unwoven PGA in mechanical performance and at the same time degraded more slowly.
The cellular responses to these materials, as well as their individual degradation profiles, appear to be very different. Lu et al. (Lu HH et al. 2005) compared scaffolds based on three different materials, PGA, poly-Llactic acid (PLLA) and PLGA. Although the PGA-based scaffolds showed the highest initial strength, they suddenly lost mechanical strength owing to the bulk degradation profile of PGA, and this resulted in a matrix disruption and a loss of integrity. Regarding cellular responses, it was reported that, when using PLLA and PLGA, the morphology of attached cells resembled that of tendons and ligaments, whereas the best cell proliferation was reported for surface-modified PLLA scaffolds.
Despite their advantages, polyesters also suffer from several limitations. First, owing to their hydrophobic nature, poly-a-hydroxyesters do not support a high level of cell adhesion (Wan YQ et al. 2003) which is the initial and crucial step to engineer functional tendons.
Fortunately, this limitation can be overcome by means of surface modification with adhesive agents such as fibronectin (Qin TW et al. 2005)
Second, although degradation products of PGA, PLA and PLGA are natural metabolites, they are also acidic. The presence of these metabolites in large concentrations can therefore give rise to significant systematic or local reactions (Bostman OM et al. 2000)
When the sizes of scaffolds are smaller, the occurrence of such adverse biological reactions is greatly reduced. Therefore, in general, polyesters are more used for repair of smaller defects, which need smaller scaffolds.

2.3 Polysaccharides scaffolds

Polysaccharides have also been applied in the field of soft tissue engineering, and chitosan in particular has been used to regenerate tendons.

Chitosan, a deacetylation product of chitin, is a linear polysaccharide composed of randomly distributed b-(1–4)-linked D-glucosamine (the deacetylated unit) and N-acetyl-D-glucosamine (the acetylated unit), it is hydrophilic and exhibits good cell adhesion and proliferation characteristics (Suh JKF & Matthew, HWT 2000)

Moreover, the N acetylglucosamine present in chitosan is a structural feature that is also found in glycosaminoglycan, which is involved in many specific interactions with growth factors, receptors and adhesion proteins. Chitosan as a glycosaminoglycan analogue might therefore also exhibit similar bio-functionality. Furthermore, chitosan can create highly porous structures that make it especially suitable for a scaffolding material used in tendon tissue engineering (Kumar MNVR et al. 2004)

The bio-functionality of chitosan, such as supporting of cellular attachment and proliferation, and the ability to induce cells to produce ECM has been demonstrated. In a study conducted by Bagnaninchi et al. (Bagnaninchi et al. 2007), porous chitosan scaffolds with microchannels were designed to engineer tendon tissues.

Hyaluronan (HA) is a uniformly repetitive linear GAG composed of disaccharides of glucuronic acid and N-acetylglucosamine: [-b (1,4)- GlcUA- b (1,3)-GlcNAc-]n (Toole BP 2001) It is an essential component of ECM. Anionic hyaluronan interacts with other macromolecules, such as link proteins and proteoglycans, to facilitate tissue morphogenesis, cell migration, differentiation and adhesion (Toole BP 2001), whereas cationic chitosan can elicit electrostatic interactions with anionic glycosaminoglycans and other negatively charged species. (Kumar MNVR et al. 2004)

Hybridization of hyaluronan and chitosan is expected to augment the mechanical properties and bioactivities of tendon tissue engineering scaffolds. Funakoshi et al. (Funakoshi et al. 2005a) demonstrated that scaffolds composed of hybridized chitosan–hyaluronan exhibited enhanced mechanical competence. In another study, Funakoshi et al. (Funakoshi et al. 2005b) reported that the chitosan–hyaluronan scaffold improved the biomechanical properties of the regenerated tendon tissue in the rotator cuff and bolstered production of collagen type I.

Alginate, another type of polysaccharide that can be used for hybridization with chitosan, is an anionic polysaccharide composed of homopolymeric regions of glucuronic acid and mannuronic acid interspersed with mixed sequences (M-G blocks). Because it contains D-glucuronic acid as the main sugar residue in the repeat unit, alginate is often considered to have similar biological activity to glycosaminoglycans. However, owing to its anionic nature, cell adhesion to alginate is often unsatisfactory (Rowley JA et al. 1999) (Genes NG et al. 2004)

Adding cationic chitosan to alginate would augment the bio-functionality of the scaffold because the ionic interactions between alginate and chitosan are expected to facilitate retaining and recruiting of cells and growth factors, as well as cytokines (Madihally SV & Matthew HWT 1999) (Hsu SH et al. 2004)

It was reported that an alginate–chitosan hybrid scaffold showed significantly enhanced cell adhesion to tenocytes. (Majima et al. 2005)

2.4 Decellularized scaffolds

In order to be utilized successfully as a biomaterial, native extracellular matrix must first be decellularized to remove any allogenic cells and to prevent adverse immunological reactions. Native scaffolds are bioactive and promotes cellular proliferation and tissue ingrowth.

An ideal cell removal method would not compromise graft structure and mechanical properties.

Cartmell JS & Dunn MG (Cartmell JS & Dunn MG 2000) compared the effects of three extraction chemicals [t-octyl-phenoxypolyethoxyethanol (Triton X-100), tri(n-butyl)phosphate (TnBP), and sodium dodecyl sulfate (SDS)] on tendon cellularity, structure, nativity, and mechanical properties.

Treatment with 1% SDS for 24 h or 1% TnBP for 48 h resulted in an acellular tendon matrix with retention of near normal structure and mechanical properties, cell removal using SDS and TnBP, suggested these treatments are potentially useful for removing cells from tendon allografts or xenografts without compromising the graft structure or mechanical properties.

In order to function as a living tissue, it is essential that the acellular scaffold is recellularized either in vivo or in vitro prior to implantation, so that remodeling of the scaffold to maintain the correct ultrastructural and physical properties can occur. Recellularization in vitro allows for further conditioning of the graft prior to implantation, and hopefully a more successful outcome.

Several approaches have been developed to reseed scaffolds that are used in tissue engineering, including static culture, pulsatile perfusion and centrifugal force. However, the recellularization in most cases was not homogenous or required large numbers of cells.

Harrison RD Gratzer PF (Harrison RD & Gratzer PF 2005) developed a decellularized bone-anterior cruciate ligament, demonstrating that Triton-X-and TnBP-treated ligaments were more receptive to cellular ingrowth than SDS-treated samples.

Woods T & Gratzer PF (Woods T & Gratzer PF 2005) reported that TnBP treatment slightly decreased the collagen content of the anterior cruciate ligament, but did not alter its mechanical properties.

In a study, Ingram JH et al. (Ingram JH et al. 2007) have decellularized a porcine patella tendon scaffold with hypotonic buffer, 0.1% (w/v) sodium dodecyl sulfate (SDS), then used an ultrasonication treatment in order to produce a microscopically more open porous matrix; cells seeded onto the fascicular scaffolds penetrated throughout the scaffold and remained viable after 3 weeks of culture.

Deeken CR et al. (Deeken CR et al. 2011) decellularized the central tendon of the porcine diaphragm with several treatments but only 1% TnBP was effective in removing cell nuclei while leaving the structure and composition of the tissue intact.

3. Cells

An important prerequisite for current tendon engineering is the successful isolation and selection of functionally active cells, the cells have to retain the ability to proliferate rapidly *in vitro* to provide adequate numbers for *in vivo* implantation.

The most common cell types employed are fibroblasts, tenocytes and mesenchymal stem cells/marrow stromal cells. (Doroski DM et al. 2007)

The main cell type found in tendon tissue is the fibroblast, which is responsible for secreting and maintaining the extracellular matrix. Hence, fibroblasts are the predominant cell type used for tissue engineering applications. (Doroski DM et al. 2007)

Two different fibroblast populations can be found in the tendon: the elongated tenocytes and the ovoid-shaped tenoblasts.(Li F et.al 2008) Elongated tenocytes proliferate well in culture and have optimal morphology in terms of expression of collagen type 1, which is a major component of normal tendons. (Li F et.al 2008) Tendon cells are usually isolated from

human tendon samples by tissue dissociation techniques.(Bagnaninchi PO et al. 2007) (Yao L et al 2006) (Cao D et al 2006)

After two or three cell culture passages and before they lose their phenotype, they are seeded into collagen gels or into scaffolds at an appropriate cell density (10^6 cells/mL). (Bagnaninchi PO et al. 2007) (Yao L et al 2006) (Cao D et al 2006)

Anyway the use of tenocytes have some drawbacks such as limited availability of donor sites for cell harvest, the requirement for lengthy *in vitro* culture to expand the number of cells, and donor-site morbidity limit the practicality of this technique. (Hankemeier S et al. 2005) (Awad HA et al. 2000)

Stem cells may represent the ideal source for tendon engineering.There are 2 types of stem cells: embryonic stem cells and adult stem cells, embryonic cells are totipotent, but their practical use may be limited because of ethical issues and concerns regarding cell regulation. Adult stem cells, also known as mesenchymal stem cells, show excellent regenerative capacity, the ability to proliferate rapidly in culture, and the ability to differentiate into a wide variety of cell types.(Gao J and Caplan AI 2003) (Alhadlaq A & Mao JJ 2004) (Bosnakovski D et al. 2005) (Grove JE et al. 2004)

The ability of human marrow derived adult mesenchymal stem cells that have tendinogenic differentiation already has been documented in several studies: mesenchymal stem cells can be stimulated to differentiate into fibroblasts when exposed to mechanical stress, (Ge Z et al. 2005) and their rates of proliferation and collagen excretion have been shown to be higher than those of fibroblasts, so they may be a viable alternative to fibroblasts. (Li F et.al 2008)

The ideal source of autologous stem cells would be one that is easy to obtain, results in minimal patient discomfort, and provides cell numbers substantial enough to obviate extensive expansion in culture.

Studies have shown that raw adipose tissue contains a population of adult stem cells that can differentiate into bone, fat, cartilage, or muscle in vitro.(Lee RH et al. 2004) (Zuk PA et al. 2001) (Lee JA et al. 2003)

These adipose-derived stem cells are easily accessible and unlike marrow are available in large quantities with acceptable morbidity and discomfort associated with their harvest.

The autologous nature of these stem cells together with their putative multipotentiality and ease of procurement may make these cells an excellent choice for many future tendon-engineering strategies and cell-based therapies.(Young RG et al. 1998)

Tissues treated with these cells showed a markedly larger crosssectional area and contained collagen fibers that were better aligned than those in matched controls. (Awad HA et al. 1999)

Compared with their matched controls the MSC-mediated repair tissue showed marked increases in maximum stress, modulus, and strain energy density. Morphometrically,there were no marked differences in microstructure between the experimental and the control sides.

Kryger et al.(Kryger et al. 2007) compared tenocytes and mesenchymal stem cells for use in flexor tendon tissue engineering. They studied four candidate cell types for use in reseeding acellularised tendon constructs. Specifically, they compared epitenon tenocytes, tendon sheath fibroblasts, bone marrow-derived mesenchymal stem cells (BMSCs), and adipoderived mesenchymal stem cells (ASCs) with respect to their in vitro growth characteristics, senescence and collagen production, as well as the viability of reseeded constructs.(Kryger et al. 2007) They also studied the in vitro viability of tendon constructs after reseeding and after *in vivo* implantation in a clinically relevant model of rabbit flexor tendon grafting. Results showed that epitenon tenocytes, tendon sheath cells, bone marrow

and adipo-derived stem cells have similar growth characteristics and can be used to successfully reseed acellularized tendon grafts.(Kryger et al. 2007) Constructs using the four cell types were also successfully implanted *in vivo* and showed viability after six weeks following implantation. (Kryger et al. 2007) The most relevant novel finding is that adipo-derived mesenchymal stem cells showed higher proliferation rates at later passages when compared with epitenon tenocytes. adipoderived mesenchymal stem cells have been shown to have multipotency and may be driven toward tenocyte differentiation when seeded into tendon constructs and exposed to the appropriate environment and mechanical forces. (Kryger et al. 2007) As confirmed by immunocytochemistry analysis, these stem cells also produce collagen, suggesting that they would contribute to *in vivo* tendon matrix remodeling. In conclusion, these results suggest that adipoderived mesenchymal stem cells have a practical advantage when compared with epitenon tenocytes and sheath fibroblasts, given that it is easier to harvest large amounts of fat tissue.

4. Local delivery of growth factors and gene therapy

Numerous growth factors that promote soft-tissue regeneration such as platelet-derived growth factor (PDGF), epidermal growth factor, fibroblast growth factor, insulin-like growth factor-I, bone morphogenetic proteins (BMPs) 2 to 7, growth and differentiation factors 5 to 7, and transforming growth factors 1 to 3 have been studied. (Hankemeier S et al. 2005) (Lou J et al. 2001) (Abrahamsson SO et al. 1991) (Rickert M et al. 2001) (Wang XT 2004) (Hsu C & Chang J 2004).

The results of *in vitro* studies have shown that growth factors can promote cell proliferation and protein synthesis (Fu SC et al. 2003) (Jann HW et all 1999)Injured tendons treated with growth factors show improved mechanical properties. (Aspenberg P & Forslund C 1999) (Zhang F et al. 2003) (Chan BP 2000).

Gene therapy in tendon engineering is an attractive new approach to the treatment of tendon lacerations.

The challenge is to define optimal cellular targets and to identify genes that are of therapeutic value and vectors that can deliver these genes with minimal side effects and maximal efficiency and durability.

A variety of gene transfer techniques can be used to maintain local concentrations of growth factor at the repair site by continuous expression of the exogene. (Dai Q et al 2003)

Vectors that enable the uptake and expression of genes into target cells are grouped into viral and nonviral. Viral vectors are viruses that are deprived of their ability to replicate and into which the genetic material can be inserted. These vectors are effective because host cells into which they introduce their genetic material form part of their normal life cycle.(Lou J et al. 1996)

Nonviral vectors such as liposomes are less pathogenic because of the absence of viral proteins, but are less efficient than viral vectors in the transfer of DNA to cells. (Jayankura M et al. 2003)

For effective tissue regeneration it is important to develop methods that will deliver genes to the site of tendon injury. (Lou J 2000) (Hildebrand KA et al. 2004)

Two main strategies for gene transfer using vectors can be envisioned: (1) *in vivo* transfer with a vector that is applied directly to the relevant tissue and (2) removal of cells from the body, transfer of the gene *in vitro*, and after an additional intermediate step that involves culture of the cells, reintroduction into the target site.

Direct gene transfer is less invasive and technically easier than transfer to cells *in vitro* because treatment can be started during the acute phase of injury. One disadvantage is the possibility of nonspecific infection of cells that are adjacent to the injury site. This risk may be complicated further by the fact that owing to the amount of extracellular matrix present and the relative few cells, a vector with high transgenic activity is needed to transfer the gene effectively to enough cells *in vivo*.

Furthermore BMP-12 gene transfer into a complete tendon laceration in a chicken model produced a 2-fold increase in tensile strength and stiffness of repaired tendons. (Lou J et al. 2001)

Recombinant adenovirus-expressing green fluorescent protein or BMP-13 injected into rabbit tendons showed efficient dosedependent transgene expression in all samples at 12 days after injection, although lymphocytic infiltration was noticed at the injection sites with the highest dose of virus, suggesting that injected adenoviral vectors elicit a local inflammatory response.

Although the *ex vivo* indirect technique includes the additional step of preparing cells and maintaining them *in vitro*, it provides a greater margin of safety because modified cells can be tested *in vitro* before administration, and viral DNA that is carried by these cells is not administered directly to the host cells. In addition gene transfer *in vitro* allows the selection of cells that express the trans-gene at high concentrations by using a selectable gene.

A variety of viral vectors such as adenovirus, retrovirus, adeno-associated virus, and liposomes have been evaluated for their ability to deliver genes to tendon, ligament, and meniscal cells. (Gerich TG et al. 1997)

Although adenovirus was the most effective vector in short-term experiments, transgene expression was transient; although the retrovirus gave lower initial transduction efficiencies, the percentage of transduced cells could be increased with a selectable marker gene.

The transfer of PDGF-B DNA to tenocytes increased the expression of PDGF and type I collagen gene expression in cultured tendon cells.Bone-marrow mesenchymal cells transfected with BMP-12 complementary DNA are placed into muscle in nude mice induced a neo-tendinous tissue. (Wang XT 2004)

Furthermore type I collagen synthesis was increased in tendon cells that were transfected with BMP-12 complementary DNA. Platelet-derived growth factor increased flexor tendon cell proliferation *in vitro.* (Thomopoulos S et al. 2005)

The PDGF and insulin-like growth factor-I transduced cells stimulated collagen and DNA synthesis in adjacent tendon cells. (Thomopoulos S et al. 2005)

The polymer that was seeded with tendon cells *in vitro* was used to repair a rotator cuff tear and histological studies showed that the tissue-engineered construct restored tendon with nearly complete repair of the tear. The restoration of normal tendon histology with longitudinally aligned collagen fiber bundles in the experimentally treated animals was shown.

5. Tendon engineering by the application of mechanical load

In vitro tissue development may include the application of mechanical loading to precondition the engineered tissue for the *in vivo* mechanical environment. Mechanical stress plays a significant role in modulating cell behavior and has driven the development of mechanical bioreactors for tissue engineering applications.(Ingber DE 2006) (Barkhausen T et al. 2003) (Brown RA et al. 1998) (Wang JH et al. 2004)

Tendons transmit force from the muscle to the bone and act as a buffer by absorbing external forces to limit muscle damage. Tendons exhibit high mechanical strength, good flexibility, and an optimal level of elasticity to perform their unique role. Tendons are visco-elastic tissues that display stress relaxation and creep. The mechanical behavior of the constituent collagen depends on the number and types of intramolecular and intermolecular bonds.

Experiments have confirmed cell growth and function would be controlled locally through physical distortion of the associated cells or through changes in cytoskeletal tension. Moreover, experimental studies have demonstrated that cultured cells can be switched between different fates including growth, differentiation, apoptosis, directional motility or different stem cell lineages, by modulating cell shape. (Barkhausen T et al. 2003) (Brown RA et al. 1998) (Wang JH et al. 2004) (Schulze-Tanzil G et al. 2004)

Externally applied cyclic strain under in vitro conditions has enormous effects on various functions of tenocytes, such as their metabolism, proliferation, orientation and matrix deposition (Screen HRC et al. 2005) (Yamamoto E et al. 2005)

Kessler et al. (Kessler et al. 2001) demonstrated that collagen fibres and tendon cells can be oriented along the direction of the stress and can upregulate synthesis of tissue inhibitor matrix metalloproteinases-1 and -3 as well as of collagen type I, the main component of tendinous extracellular matrix.

It is known that cyclic strain can affect cell morphology and induce uniaxial cellular alignment. It was observed that cyclic strain stimulation enhanced the cellular alignment and changed the cellular shape.

Other experiments have demonstrated the beneficial effects of motion and mechanical loading on tenocyte function. Repetitive motion increases DNA content and protein synthesis in human tenocytes in culture.(Almekinders LC et al. 1995) (Sharma P and Maffulli N. 2005) Even fifteen minutes of cyclic biaxial mechanical strain applied to human tenocytes, results in improved cellular proliferation.(Sharma P and Maffulli N. 2005) (Zeichen J et al. 2000)

Moreover, *in vitro* cyclic strain allows an increased production of TGF-β, FGF and PDGF by human tendon fibroblasts.(Bagnaninchi PO et al. 2007) (Slutek M et al. 2001)

Cyclic stretching of collagen type I matrix seeded with MSCs for 14 days (8 h/day) resulted in the formation of a tendon-like matrix. (Bagnaninchi PO et al. 2007) (Zeichen J et al. 2000)

Expression of collagen types I and III, fibrinonectin and elastin genes was found to have increased when compared with nonstretched controls in which no ligament matrix was found. (Bagnaninchi PO et al. 2007) (Yang G et al. 2004)

The model reproduces *in vivo* tendon healing by preventing differentiation of tenocytes into fibroblasts.

In animal experiments, mechanical stretching has improved the tensile strength, elastic stiffness, weight and cross-sectional area of tendons.(Sharma P & Maffulli N 2005) (Kannus P et al. 1992) (Kannus P et al. 1997) These effects result from an increase in collagen and extracellular matrix network syntheses by tenocytes. Application of a cyclic load to wounded avian flexor tendons results in the migration of epitendon cells into the wound.(Sharma P and Maffulli N 2005) (Tanaka H et al. 1995)

Also, Qin et al. (Qin et al. 2005) found that cyclic strain promotes cell proliferation, matrix deposition and increased collagen production. In another study, Juncosa-Melvin et al. (Juncosa-Melvin et al. 2007) showed that the application of cyclic strain elevated the gene expression levels of collagen type I. Finally, cyclic strain can enhance mechanical

competence of the regenerated tendons (Juncosa-Melvin et al. 2006). The authors of this study found that values for maximum force, linear stiffness, maximum stress, and linear modulus for repaired tendons were close to those of natural patellar tendon. In terms of restoration of key biomechanical parameters, these constructs appear to be the best engineered tendons obtained so far.

Clinical studies have shown the benefit of early mobilization following tendon repair, and several postoperative mobilization protocols have been advocated.(Buckwalter JA 1996) (Chow JA et al 1988) (Elliot D et al. 1994) The precise mechanism by which cells respond to load remains to be elucidated. However, cells must respond to mechanical and chemical signals in a coordinated fashion. For example, intercellular communication by means of gap junctions is necessary to mount mitogenic and matrigenic responses in ex vivo models. (Sharma P & Maffulli N 2005)

Duration, frequencies and amplitude of loading directly influence cellular response and behavior in many other tissues. Understanding the physiological window for these parameters is critical and represents future challenges of research in tendon tissue engineering.

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These contribution books collect reviews and original articles from eminent experts working in the interdisciplinary arena of biomaterial development and use. From their direct and recent experience, the readers can achieve a wide vision on the new and ongoing potentials of different synthetic and engineered biomaterials. Contributions were not selected based on a direct market or clinical interest, than on results coming from very fundamental studies which have been mainly gathered for this book. This fact will also allow to gain a more general view of what and how the various biomaterials can do and work for, along with the methodologies necessary to design, develop and characterize them, without the restrictions necessarily imposed by industrial or profit concerns. The book collects 22 chapters related to recent researches on new materials, particularly dealing with their potential and different applications in biomedicine and clinics: from tissue engineering to polymeric scaffolds, from bone mimetic products to prostheses, up to strategies to manage their interaction with living cells.

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