Therapeutic strategies for tendon healing based on: novel biomaterials, factors, and cellsFrom *In Vitro* studies to *In Vivo* Application

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Abstract.

The repair of tendon ~~and ligament~~ injuries still presents a major clinical challenge to orthopaedic medicine. T~~Soft tissues like t~~endons, like some other tissues, ~~and ligaments~~ are poorly vascularized and heal slowly. In addition, healing often leads to the formation of fibrous and scarry tissue which lacks the original flexibility and biomechanical properties. Therefore, the treatment of tendon injuries is challenging. Here, we will give a brief overview on the structure and composition of tendons, pathological states of tendon and natural healing, was well as on therapeutic options. We focus in particular on biomaterials that have been specifically developed or suggested for the successful repair of tendon ~~and ligament~~ injuries. In addition, we also shortly review factor- and cell-dependent strategies to heal tendon and ligament disorders. Although short, we hope that this review will be helpful for especially for those readers who are new to the field of tendon tissue engineering.

I. Structure and composition of tendons~~ and ligaments.~~

Ligaments and tendons, the flexible structures that bind together the musculoskeletal system, are extraordinarily strong in resisting tensile loads. Both are composed of dense fibrous connective tissue but they differ in morphology and function ~~as described below~~. Due to substantial biochemical differences they also differ in their biomechanical properties. On average, tendons contain fewer cells and are less metabolically active than ligaments. Their modest metabolic rate and well-developed anaerobic energy generation enable tendons and ligaments to bear loads and maintain tension for extended time periods.

A characteristic feature of tendons ~~and ligaments~~ is the wavy crimp pattern that the collagen fibres demonstrate and which is seen in histological sections: In the absence of load, a regular sinusoidal wave pattern is observed in longitudinal sections. Upon stretching, the crimp pattern disappears and the fibres become straight. Upon release, the crimped pattern re-appears. T~~Both t~~endons and also ligaments exhibit a hierarchical structure which is based on the organisation of collagen type I, a triple-helical molecule: The soluble tropocollagen molecules spontaneously self-assemble after secretion and cross-linking into collagen microfibrils. Those microfibrils arrange themselves into larger units of subfibrils and fibrils. The fibrils then gather into collagen fibres that can be detected by light microscopy. Figures depicting the structural hierarchy of ligaments and tendons are depicted in a number of recent reviews to which we refer at this place: see [1].

The key difference between tendons and ligaments is their anatomical position (cf. below) rather than their histology or gene expression profile [2]: They express many similar proteins including extracellular matrix and soluble proteins and transcription factors like TGF-β, scleraxis, decorin and other proteoglycans, tenomodulin, fibromodulin, biglycan, and the collagens [3]. Due to a lack of specific "markers" many papers do not distinguish between these two tissues. To generate functional and self-renewing tendon tissue, the normal processes of tendon development must be elucidated in more detail. In particular, we need to understand which stem cell populations in the body are able to form tendon and ligament, how they can be directed to do so in culture without simultaneously forming other skeletal tissues, how their growth is controlled, and how normal cell turnover can be re-established and maintained in the tissue-engineered tendon.

Ligaments connect bones to each other in order to restrict their relative motions and they also support organs. According to this definition, the patellar tendon in fact is a ligament. ~~The major function of ligaments is mechanical, as they passively stabilize joints and help in guiding those joints through their normal range of motion when a tensile load is applied . Capsular ligaments act as mechanical reinforcements, while extra-capsular ligaments join together and provide joint stability. A second and less appreciated function of ligamentous tissue is proprioception via stretch-sensitive mechanoreceptors. They serve to protect, e.g., the knee, from extremes of motion.~~

~~Ligaments are composed of 55 - 65 % water. Additional components are 70 - 80 % collagen (type I predominant, some type III), elastin 10 - 15 %, and proteoglycans, glycosaminoglycans, and glycoproteins (e.g. fibronectin, thrombospondin) making up the remaining 1 - 3 % of the dry weight. Fibrillar collagen type I gives ligament its high tensile strength and is synthesized by specialized cells called fibroblasts and fibrocytes.~~

Tendons link muscles to bone and thus provide a connecting link transmitting forces developed by muscle contractions. ~~Tendons originate in muscle (musculo-tendinous junction) and insert into bone (osteo-tendinous junction, enthesis).~~ On one hand, a single tendon (e.g. the Achilles) can focus the action of several muscles onto one bone, on the other hand, a single muscle (e.g. the tibialis posterior) can spread its action through several tendons that attach to different bones. Individual tendons vary a lot in their shape, size and function, representing adaptations to specialized functions and this may need to be considered for therapeutic treatments. Tendons also function in proprioception. A third function of tendons is energy storage in form of elastic strain energy in stretched tendons.

~~The composition of tendons is similar to ligaments.~~ 70 % of the tendon mass is water. Within the dry mass, the~~ir~~ collagen content ~~is somewhat higher,~~amounts to between 75 and 85 %, with~~and~~ 95 % being type I collagen, 5 % being type III and/ of V. Their elastin content of less than 3 % dry weight is significantly lower than in ligaments, and proteoglycans, glycosaminoglycans, and glycoproteins are about 2 % [5]. Tenoblasts are immature tendon cells. They are spindle-shaped, with numerous cytoplasmic organelles reflecting their high metabolic activity. As they age, tenoblasts become elongated and transform into tenocytes. These have a lower nucleus-to-cytoplasm-ratio than tenoblasts, with decreased metabolic activity, are interspersed between the collagen bundles and lie along the long axis of the tendon [6].

The structural hierarchy of tendons resembles ligaments but is more elaborate since collagen fibres are arranged in discrete packets called fibres or subfascicles (primary fibre bundles) and fascicles (secondary fibre bundles) that are bound together by the endotenon. The endotenon is surrounded by and continuous with the epitenon which surrounds the whole tendon. Surrounding the epitenon superficially, another thin layer called paratenon is present which allows free movements within the surrounding tissue. Epi- and paratenon together constitute the peritenon. Long tendons such as the digital flexor tendon that are present in areas subjected to increased mechanical stress - specifically in the hands and the feet - are additionally enclosed in a synovial sheath that gives lubrication and enhances gliding.

T~~As mentioned, t~~here are two junctions (attachment sites) for each ~~ligament and~~ tendon. These junctions need to resist forces of hundreds of Newtons and are also highly prone to injury. The different physical, structural/biochemical and mechanical properties of the tissues involved in the formation of these junctions need to be considered particularly during the development of therapeutic options.

At the myotendinous junction (MTJ) tendinous collagen fibrils are inserted into deep recesses formed by myocyte “finger-like” processes, allowing the tension generated by intracellular contractile proteins of muscle fibres to be transmitted to the collagen fibrils. The folding of the tissues at the MTJ strongly increases the contact area between the muscle and the tendon fibres and reduces the tensile stress exerted on the tendon during muscle contraction. Satellite cells (muscle stem cells) are present even in adult MTJ [7].

The osteotendinous junction (OTJ, enthesis) occurs in two different shapes. A fibrocartilaginous (direct) enthesis (e.g. on epiphyses of long bones, on the short bones of the wrist and ankle: [8]) is composed of four zones: a dense fibrous connective tissue tendon ~~or ligament~~ zone, uncalcified fibrocartilage, mineralized fibrocartilage, and bone. The outer border of calcification is indicated by a so-called tidemark with basophilic nature, similar to the tidemark found in articular cartilage. The tendon zone contains fibroblasts whereas fibrocartilage cells prevail in the two fibrocartilage zones and osteoblasts/osteocytes in the bone. The fibrocartilage cells synthesize an extracellular matrix that is rich in aggrecan and collagen type II, both of which are typical of articular cartilage. Their function is to trap and hold water, thereby resisting compression and dissipating stress at the tendon~~/ligament~~ – bone interface. As in articular cartilage, the fibrocartilage cells lie isolated within the matrix which creates a barrier to communication between the osteoblasts/osteocytes in the bone and the fibroblasts in the tendon [7]. A fibrous (indirect) enthesis (e.g. in the meta- or diaphyses of long bones: [8]) lacks both the fibrocartilage intermediate zone and is made up of tendon and bone zones only.

II. Pathology and Natural Healing.

Tendon and ligament injuries account for considerable morbidity both in sport and the workplace, and often prove disabling for several months. Chronic problems caused by overuse of tendons account for about 30 % of all running-related injuries, and the prevalence of elbow tendinopathy in tennis players can be as high as 40 % [6]. The social and economical burden associated with these medical conditions both in athletes and working and elder people calls for greater understanding of basic tendon biology and expanding research on translational applications. Tendon injuries can be acute or chronic and are caused by intrinsic or extrinsic factors, either alone or in combination. Intrinsic factors are: age, gender, biomechanics, the presence or absence of systemic diseases, and probably also genetic factors as discussed by [9]. Extrinsic factors include: physical load, environment, occupation, and training. In acute trauma, extrinsic factors predominate, whilst in chronic cases, intrinsic factors also play a role. Overuse injuries generally have a multi-factorial origin.

The terms "tendonitis**"** or "tendinitis" are a description of "tendon inflammation" and as such are not very appropriate: Although inflammatory conditions of tendon ~~and ligament~~ are possible they are not the principal cause of tendon injury, and therefore, traditional treatment modalities aimed at controlling inflammation may not be optimal. Instead, the most common disorders involve degenerative processes due to an inability to resist the loads that the tendon is subjected to. Therefore, in general, these conditions are more of a degenerative

than inflammatory nature, and inflammatory reactions occur essentially only subsequent to the damage. Therefore, the term "tendinosis" (a degenerative tendon without accompanying inflammation) to describe the tendon degeneration in tendinopathy (no implication for pathology is included in this term) would be more adequate [9]. "Tenosynovitis" implies imflammatory changes in synovial sheaths but is a term commonly used in any tendon sheath disorder, particularly common in the hand and wrist [10].

Certain tendons are especially vulnerable to degenerative pathology, including the Achilles, patella, parts of the rotator cuff (supraspinatus), forearm extensors, biceps brachi, and tibialis posterior tendons. ~~In the knee, the four major stabilizing ligaments, i.e. the anterior cruciate ligament (ACL), the posterior cruciate ligament (PCL), the medial collateral ligament (MCL), and the lateral collateral ligament (LCL) are prone to injury. Especially, it is not uncommon for multiple knee structures to become hurt. The simultaneous injury of ACL, MCL and LCL is a common "triad".~~ Injuries at OTJ ("enthesopathies") can result from repetitive strains from metabolic, endocrine, and inflammatory diseases. They are also common, usually accompanied by muscle weakness and include tennis elbow, golfer´s elbow, and jumper´s knee [10].

The natural healing process can be divided into three overlapping phases: First, the inflammatory stage within hours to a few days, second, the remodeling stage within a few days when tenocytes gradually migrate to the wound, and type III collagen synthesis is initiated and third, the modeling stage after about 6 weeks. Phase 3 can be divided into a consolidation and a maturation phase during which the healing tissue is resized and reshaped. The molecular events during these stages are well characterized. However, the use of selected growth factors or extracellular matrix molecules singly or in combination did not yield notable therapeutic effects.

Although there is incessant cell renewal and matrix turnover at a relatively slow rate in tendons ~~and ligaments~~ their hypovascularity, hypocellularity and the low metabolic rate entail slow healing after injury [11]. At the end of natural healing, the biochemical and mechanical properties never match those of intact tendon~~/ligament~~. Obviously, the cellular and molecular signals that lead to formation of the native insertion site during embryogenesis and development are not recapitulated during tendon healing [3;12] resulting in scar formation with collagen III prevailing. In addition, tendon ~~and ligament~~ damage is an important cause of successive joint instability and may progress into early onset of osteoarthritis, pain, disability and eventually the need for joint replacement surgery. Because the healing responses are different in ligaments and tendons after injury, the consequences and treatments are tissue-

and site-specific. Results of non-surgical managements (like rest, compression, cooling, corticosteriod injection, ultrasound or laser treatment) mainly provide pain relief. When they fail, surgery is required [3]. Surgical reconstructions including tissue autografts (using, e.g. patellar or hamstring tendons), artificial prostheses, or graft-augmentations are regularly performed by clinicians but the long-term results are not satisfactory since the operated tissue is prone to re-injury besides the numerous complications accompanying surgeries [13]. In summary, the management of tendon~~/ligament~~ injury poses a considerable challenge since the current treatment options mainly allow for a replacement or a minimization of the tissue damage which results in pain relief or replacement of the pathological tissue but they do not heal the damage itself. Fully functional tissue ("regeneration") is never achieved.

III. Therapeutic Options.

Despite all efforts, current treatment modalities are suboptimal, and alternative strategies are required. Our knowledge of the mechanisms regulating tendon ~~and ligament~~ development during embryogenesis is scarce. Even more limited is our knowledge concerning the molecular pathways that are involved in tendon ~~or ligament~~ regeneration in the adult. In addition, there are clear limitations in defining optimal conditions for tenogenic differentiation in vitro and markers to assess a successful differentiation into tendon~~/ligament~~ forming cells [3]. This complicates evaluation of in vitro and in vivo studies. Nevertheless, to obtain scientific and clinical progress a combination of knowledge from different scientific disciplines including molecular biology/genetics, biochemistry, biomechanics, nanotechnology, engineering, and others will advance tissue engineering and regenerative medicine (TERM) of tendon ~~and ligament~~. These include: novel extracellular matrix ("biomimetic") bioscaffolds, delivery of growth factors, (stem) cell-derived therapy, gene-therapeutic approaches based on vehicles encoding selected factors, and advanced bioreactors with application of mechanical load. We will here review some recent developments in these topics. Although much of this work is promising, further investigations are necessary to achieve consistent results that can also be translated into the clinics. As far as the use of cells and stem cells is concerned, regulatory issues may apply that further complicate routine clinical applications. At current, it is difficult to predict which of the individual strategies will have the most beneficial input in the future.

A. Scaffolds, ~~and~~ biomimetic materials, and grafts.

Depending on their origin, scaffolds can be fully natural, fully synthetic, or a mixture of both. Another method of classification distinguishes resorbable/biodegradable from non-resorbable/permanent scaffolds depending on the chemical nature of the scaffold´s components. The ideal scaffold should have some key properties that can be summarized by

the following catchwords: biodegradability, biocompatibility, processability, mechanical strength, and biofunctionality. In terms of biodegradability, the scaffold shall provide temporary support until the neotissue has been built up into a state that will be able to function and bear loads. In vivo-degradation will subsequently obviate surgical removal. Biocompatibility means absence of an immunological response, interaction with and ideally integration into the host tissue. Processability implies the possibility of mimicking native tissue and addressing tissue needs. The scaffold should be able to bridge any complex three-dimensional anatomical defect. Porous scaffolds enhance tissue regeneration by delivering biofactors. Pore diameter is important in facilitating cell migration, proliferation, and growth factor movement. It is important to obtain the right balance between tissue regeneration and the mechanical properties of the scaffold. Whilst smaller pores are inefficient, larger pores can compromise the mechanical properties of the scaffold [14]. In addition, a scaffold should be easy to handle, store, and sterilize.

Biological (natural) bioscaffolds consist of protein-based extracellular matrices that are mammalian-derived tissues from human (allografts), equine, porcine and bovine (xenografts) sources [15;16] such as the small intestinal submucosa (SIS, mostly from porcine jejunum), urinary bladder membrane (UBM), pericardium or dermis (TissueMend). They are processed to remove non-collagen components while retaining the natural collagen structure (predominantly collagen I fibres) and mechanical properties [16]. Materials that have been approved by the Food and Drug Administration (FDA) include Restore™ and CuffPatch (both from SIS), OrthADAPT™ (equine pericardium), Zimmer collagen repair patch, Permacol (both porcine acellular dermal matrix), Bio-BlanketW, TissueMend (bovine dermis and bovine dermal extracellular matrix), and GraftJacket (human acellular dermal matrix) [17]. The major drawback of these commercial biological scaffolds is that their mechanical properties are significantly lower than those of normal tendons ~~and ligaments~~ [16].

The collagen matrix (90 % of dry weight) in SIS is immediately ready for graft purposes, and the extracellular proteins (elastin, laminins, fibronectins, and proteoglycans) confer an additional layer of stability to the product. SIS also contains proteins like FGF and TGF-β which support healing. However, SIS has been shown to undergo contracture *in vivo*, and the high batch-to-batch variability limits the therapeutic potential. Harvesting SIS can elicit an immunologic reaction and diminish its applicability for patients since an amplified inflammatory response can lead to tissue damage and poor wound healing.

Natural-based scaffolds (based on natural polymers) have also been investigated in tendon~~/ligament~~ repair~~generation~~: silk, fibrin, or polymers including polysaccharides and

proteins that share similar features with the natural ECM [18]. Silk–collagen hybrid scaffolds developed in a knitted sponge matrix have been described, as well as the application of alginate and chitosan hybrid fibres to support tendon fibroblast adhesion [3].

Silk-based biomaterials have obtained great attention lately since silk is a bio-degradable and bio-compatible material. Due to its biomechanical features various promising silk-dependent strategies have been devised for clinical application [19] and as a scaffold for adult mesenchymal stem cells as well [20]. Fibrin has been suggested as another promising biomaterial as matrix for tendon engineering [21] and also as a strategy to deliver stem cells [22] or growth factors [23]. Several other investigations emphasize the potential of collagen gels for tendon engineering [24-26], although, the reproducibility of this therapeutic approach may cause problems due to collagen batch variations [27].

Synthetic scaffolds can have much stronger mechanical properties than natural scaffolds but their biocompatibility is limited [16]. Non-degradable synthetic polymers with FDA approval for tendon ~~and ligament~~ repair include polyethylene terephthalate, polypropylene, and poly(tetrafluoro ethylene) [17]. Several biodegradable synthetic polymers also have obtained FDA approval. These include polylactic acid and polyglycolic acid, polycaprolactone, and polydioxanone [17]. Several polymers (both non-degradable and degradable) may support growth factor release and therefore offer even greater flexibility. Although results from studies were promising, scaffolds made of polyglycolic acid, for instance, have limited application due to their mechanical brittleness and the lack of functional groups for signalling molecules. Yet, none of these synthetic scaffolds has exceeded the natural scaffolds in performance such that novel polymers are an active field of research. One material that might be promising - poly(1,8-octanediol-co-citrate, POC) – has not yet obtained FDA approval. Synthetic polymers are in general more versatile than natural ones, enabling tailoring and controlling chemical and physical properties and structural features. They also represent a more reproducible source of raw materials and exhibit low immunogenicity. The manipulation of structural parameters in the design of scaffolds and their bioactivation, through the incorporation of soluble and insoluble signals for promoting cell activities, is likely to improve the neoformation of tissues [3]. Knitting, braiding, and electrospinning are the major techniques used for preparation of biomimetic fibrous scaffolds in tendon~~/ligament~~ tissue engineering. When seeded with cells, they spontaneously orientate along the direction of the fibres leading to abundant extracellular matrix secretion rich in collagens I and III [14].

The designation **"graft"** applies to tissue that serves as a transplant. Such grafts can be used as an alternative to scaffolds or biomimetic materials especially to replace severely damaged tendons. Patellar tendon and ACL tissues are commonly used autografts for tendinopathies. Allografts are less ideal due to the risk of disease transmission and rejection. And, both auto- and allografts are retrieved from a non-diseased site elsewhere in the body which may lead to notable morbities at the donor site.

B. Delivery of proteinaceous factors.

Different growth factors such as bFGF (fibroblast growth factor-2), TGF-β/BMPs (transforming growth factor/ bone morphogenetic proteins), PDGF (platelet-derived growth factor), epidermal growth factor (EGF), VEGF (vascular endothelial growth factor), insulin-like growth factor (IGF), growth and differentiation factors (GDFs: mainly GDF5, 6, and 7 [BMP12, 13, 14]), hepatocyte growth factor (HGF) and others have been shown to be necessary for natural healing and have also demonstrated experimental usefulness for tendon ~~and ligament~~ repair.

Few transcription factors only have been documented to be expressed specifically in tendon and/or to contribute to tendon~~/ligament~~ development. So, *Scleraxis* (*Scx*) expression is predominantly confined to tendons ~~and ligaments~~ [28;29]. It has been shown, however, that tendon progenitor cells and tendons develop in *Scx*-mutant mice [30]. A potential role of *Scx* in tendon therapy, therefore, seems limited. Nevertheless, adenoviral-mediated *Scx*-expression in MSCs seems to improve healing of tendon-bone attachment sites after rotator cuff repair [31]. The finding that *Scx* upregulates BMP4 expression in tendon cells at the site of tendon-bone insertions [32] could support the latter observation. Other investigations show that the homeodomain transcription factor *Mohawk* influences tendon morphogenesis [33]. In Drosophila tendon differentiation has been attributed to the transcription factor *Stripe*. Its vertebrate homologues *Egr1* and *Egr2*/*Krox20* seem also to exert an influence upon tendon formation [34]. In addition, the constitutive active signaling factor Smad8 (L+MH2) seems to be able to support formation of tendon-like tissue and the insertion of tendon into bone as well [35;36]. Because of the intracellular localization of all these proteins a protein transduction domain for an intracellular delivery of recombinant proteins would be needed.

Due to their limited half-life in vivo, the direct and local delivery of growth factors has limited use necessitating more advanced strategies for a sustained, safe and reproducible delivery. These include gene therapy, scaffolds, microspheres, micro- or nanocapsules as vehicles as well as coated sutures or dissolution in fibrin sealant [37]. Using appropriate measures, this will also allow for a controlled spatiotemporal release of factors and improve the long-term

stability and storage of these factors in tailored systems. Several strategies have even aimed at the simultaneous delivery of several factors. The potential of using synthetic PLGA microspheres, fibrin-heparin delivery systems, metallic porous materials and so on as well as refinement of these systems are being investigated [38]. Promising is also the application of Platelet-Rich-Plasma (PRP) as a source for various growth factors which efficiently contribute to anterior cruciate ligament healing [39;40], although, PRP alone seems not sufficient [41].

C. Cell sources for therapeutic strategies.

Autologous cells are likely to provide an optimal cell source, avoiding immune reactions or complications caused by grafts. Their use may be limited by availability and accessibility including the risk of donor site morbidity at the site of harvest. Also, the techniques for their successful expansion in cell culture are quite limited yet [42]. **Tenocytes or fibroblasts~~/ligamentocytes~~** isolated from ~~tendon/ligament such as~~ the rotator cuff [43], patellar and Achilles tendons [21;44;45] or from the tendon sheath present an obvious choice [46] that has been addressed. **Dermal fibroblasts (DFs)** are an alternative option [25;47;48]. DFs are commonly harvested by skin biopsy, which is a simple, timeless and cost-effective procedure and they are easily expanded during in vitro culture. More importantly, DFs share common characteristics with tenocytes, as both cell types are terminally differentiated cells and originated from mesoderm [3]. However, although common characteristics are shared by fibroblasts and tenocytes, it is questionable whether these terminally differentiated skin fibroblasts trans-differentiate into tenocytes in vivo. Such trans-differentiation may necessiate the expression of certain genes/factors similar to the reprogramming transcription factors used to generate induced **pluripotent stem cells**.

Clearly, pluripotent stem cells (induced pluripotent or embryonic) present an alternative choice. They will, however, necessitate the development of careful differentiation strategies towards tenocytes. As mentioned in the introductory paragraph into subchapter III, many molecular and phenotypical traits making up true tendon cells are unknown at present. Therefore, the development of such strategies will not be an easy task. In addition, risks of teratoma formation must be excluded for pluripotent stem cells; ethical issues relating to the origin of the embryonic stem cells would also need clarification.

Therefore~~In addition~~, a cell replacement therapy using alternative cell sources may appear reasonable. This may include other tissue-specific cells or stem cells. Adult stem cells open up the possibility to treat diverse diseases by autologous transplantation (i.e. using the patient´s own cells after ex vivo expansion), either through local application or through systemic infusion. ~~In contrast to pluripotent stem cells (embryonic stem cells, induced pluripotent cells), this avoids risk of teratoma formation, and ethical issues relating to the origin of the embryonic stem cells.~~ Several studies using adult **mesenchymal stem cells (MSCs)** have been conducted in the last few years especially in orthopaedics since they have the potential to differentiate into cell lineages of the musculoskeletal system including tendon ~~and ligament~~ cells. In addition, MSCs secrete a variety of factors ("trophic" or "paracrine actions") which help in recruitment of MSCs to sites of injury and subsequently in tissue regeneration by activation of endogenous (stem) cells. Bone marrow is the most popular source of MCSs due to its relative ease of access; alternatively, amniotic fluid, umbilical cord blood, adipose tissue, synovium, and other tissues can serve as sources for MSC isolation. Bone marrow-derived MSCs may have valid clinical utility, and various strategies have been used to coax these cells down a tenogenic lineage, including application of growth factors, ectopic expression of transcription factors, exposure to tensile loads, and high-density coculture with tenocytes [37]. It is worth mentioning that **tendon-derived stem/progenitor cells (TSPCs)** have been isolated and cultured from human, mouse, rabbit, and rat tendons [49-52]. Their abundance, however, seems to be limited and their harvest is strongly invasive as has been discussed above for tenocyte~~ss/ligamentocytes~~.

In summary, an ideal external cell source for tendon regeneration has not yet been identified and needs further investigation. In light of regulatory issues mentioned in the beginning of this sub-chapter it appears even more attractive to identify endogenous factors that would allow for a stimulation of endogenous cells to execute a tendon regeneration programn.

IV. Future aspects.

At present, there is still considerable room for improvement regarding the therapy of tendon ~~and ligament~~ injuries. Novel approaches involving the combination of mechanical stimuli, growth factors and/or hormones have to support tendon ~~or ligament~~ regeneration. Moreover, cell- and gene therapeutic strategies with tendon-derived cells or MSCs may succeed in the scar-free tendon regeneration and lead to biomechanical properties comparable with native tissue, eventually. Most important, however, improved scaffolds with an enhanced balance between stiffness and elastic compliance have to provide new therapeutic modalities for tendon ~~and ligament~~ disorders.

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