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Hoffmann, A., Gross, G.
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animal athletes
(2009) Medicine and Sport Science, 54, pp. 150-165.**

Innovative Strategies for Treatment of Soft Tissue Injuries in Human and Animal Athletes

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Short Title:

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Abstract.

Our aim is to review here some recent progress in the management of musculo-skeletal disorders. We will cover novel therapeutic approaches based on growth factors, gene therapy and cells including stem cells which may be combined with each other as appropriate. We focus mainly on the treatment of soft tissue injuries – muscle, cartilage, and tendon/ligament for both human and animal athletes. The need for innovative strategies results from the fact that despite all efforts, the current strategies for cartilage and tendon/ligament still result in the formation of functionally and biomechanically inferior tissues after injury (a phenomenon called “repair” as opposed to proper “regeneration”) whereas the outcome for muscle is more favourable. Innovative approaches are urgently needed not only to enhance the outcome of conservative or surgical procedures but also to speed up the healing process from the very long disabling periods which is of special relevance for athletes.

Introduction.

Injuries of soft tissues of the musculo-skeletal system are common and often present as long-time disabling. This situation affects individual persons and even more so professional athletes. Therefore, novel therapeutic strategies need to be developed – in particular based on regenerative medicine and tissue engineering. **Regenerative medicine** is a multidisciplinary approach to create living, functional substitutes for restoration, maintenance, or improvement of tissue or organ function that has been lost due to injury, disease, age, damage, or congenital defects – either *in vitro* for subsequent application *in vivo*, or directly *in vivo*. This field holds the promise to develop remedies for previously irreparable defects and to solve the problem of organ shortage for transplantation. One important aspect of regenerative medicine is **tissue engineering**. It includes three main elements: cells, factors or stimuli, and biomaterials. Cells (“grafts”) can be autologous or allogeneic and include stem cells where appropriate. Apart from different advantages, choice of donor cells depends on the consideration of disadvantages such as additional trauma during graft

harvesting (autograft), infection or immunorejection (allografts). The factors or stimuli are bio(re)active molecules and may be proteins like growth factors and cytokines, drugs, or others. They can be soluble, insoluble, or immobilized. The biomaterials for tissue engineering which can be derived from natural (like collagen) or synthetic sources (e. g. ceramics, polymers of lactic and glycolic acid) need to be biodegradable and must fulfill several criteria: biocompatibility, capability of integrating molecules or cells, lack of toxicity and immunogenicity, proper biomechanical characteristics, 3-dimensional structure, controlled delivery of factors and many more.

Much work within the musculo-skeletal system has been done in bone which is a highly vascularized tissue. Due to the vascularization and medical progress healing of bone after fracture often proceeds readily. In contrast, soft tissues like cartilage, tendons, and ligaments are poorly vascularized and heal slowly. And their healing ends with the formation of fibrous, scarry tissue which lacks the original flexibility and biomechanical properties.

We will here review recent progress in the management of musculo-skeletal disorders focussing specifically on innovative biological methods (“biologicals”) including gene therapy for treatment of injuries of muscle, cartilage, and tendon/ligament - for conventional human patients and also for both human and animal athletes.

I. Wound healing and soft tissue repair - current status.

After injury, all tissue healing results from a sequence of defined and consecutive steps: Hematoma formation is followed by acute inflammation, then tissue repair by proliferation of fibroblasts and remodelling take place. Ultimately, in many cases less functional tissue is generated in a process called fibrosis or scar formation as opposed to true regeneration which would restore the original functions on a molecular, cellular, functional and biomechanical level. The different stages of healing are not distinct but overlap and are mechanistically intertwined. Their exact timing depends on the type of injury and the severity of damage.

Lesions of the muscular system.

Skeletal muscle is the largest tissue mass in the body, about 40 – 45 % of total body weight, and allows for voluntary force exertion and locomotion. Lesions can be due to direct trauma (e. g. lacerations, contusions, strains, or surgical resection) or have indirect causes like ischemia or neurological dysfunction. Cancer, infectious disease, heart failure, AIDS can produce a body wasting syndrome called cachexia with severe muscle atrophy. Alternatively, muscular dystrophy denotes mostly heritable neuromuscular disorders that likewise cause muscle wasting.

Small muscle injuries like strains can heal completely but severe injuries typically result in formation of dense scar tissue that impairs muscle function. Muscle injuries in athletes account for 10 to 55 % of sports-related injuries. This frequency is highly variable and depends mainly on the type of sport. Consequently, repair of injured skeletal muscle is of major concern.

Injuries of cartilage.

Cartilage defects can be divided according to their etiology or morphology. As a result of sports injuries, focal injuries typically occur and therefore predominantly affect younger persons. These defects can be subclassified as chondral (cartilage only affected) or osteochondral (defect penetrates into the underlying bone). In older patients, degenerative chondral changes are prevalent. In contrast to bone, cartilage is a tissue with a poor intrinsic capacity for healing which results from poor vascularization (absence of blood vessels and nerve supply), lack of stem cell availability, and/or low cellular turnover and therefore, the efficiency of tissue regeneration is limited: Whereas some spontaneous but transient repair may occur with osteochondral defects since these allow access of stem cells from the vascularized bone to the lesion chondral defects do not fully regenerate.

Damage to articular cartilage in the knee is a common problem in sports; meniscal tears due to twisting or compression forces are frequent injuries. The menisci are two semilunar fibrocartilaginous structures between the tibia and femur in the knee joint. They function as shock absorbers, joint stabilizers, and joint lubricators. Only tears in the peripheral third of the menisci can heal since this part is vascularized. A special problem exists in the central avascular portion where most tears do occur since this never heals and ultimately develops into premature

osteoarthritis due to the poor regenerative capacity of articular cartilage which is a prominent example of hyaline cartilage. Sutures, arrows and staples have been used for surgical procedures but these are only partially successful in alleviating symptoms and do not prevent arthritis development. Apart from total joint arthroplasty which is associated with surgical risks and a finite life span other techniques developed for therapy include pharmacological intervention, lavage, shaving, laser abrasion, drilling or microfracture of subchondral bone to promote healing, autologous periosteal and perichondral grafting, autologous or allogeneic osteochondral transplantation, and autologous chondrocyte implantation [1]. Autologous and allogeneic grafts for cartilage now are the most common therapeutic approaches. Intense investigations have studied biomaterials on natural or synthetic basis capable of regenerating cartilage defects but so far no good solution has been identified [2] although some recent studies seem quite promising and will be discussed later in the text [3].

Injuries of tendons and ligaments.

Tendons which connect muscles to bones and ligaments that connect bones to each other are elastic collagenous tissues. They have a similar composition and hierarchical structure of collagen molecules assembling into ever larger units and their strength is related to the number and size of collagen fibrils. Since tendons and ligaments are similar in many aspects we will, for simplicity, only use the term “tendon” instead of “tendons and ligaments” in the present manuscript.

Tendon and ligament lesions can be caused by injury and trauma but also through overuse and ageing. They account for considerable morbidity both in sport and the workplace, and often prove disabling for several months. Orthopaedic surgeons see patients with blown-out knees and aching elbows daily. Successful treatment of, mostly, tendon disorders is difficult and patients often suffer from the symptoms for quite a long time. Moreover, despite remodelling, the biochemical and mechanical properties of healed tendon tissue never match those of intact tendon which is a major challenge. Tendon naturally heals but – as described above - this process results in repair only, i. e. in the formation of fibrous, scarry tissue which lacks the original flexibility and biomechanical

properties and is characterized by a homogenous distribution of smaller-than-usual-diameter collagen fibers and a higher content of collagen type V and decorin and other proteoglycans. Healed tendon therefore shows reduced performance and exhibits a substantial risk for re-injury. Due to this reason, strategies for a veritable tissue regeneration leading to enhanced elasticity and mechanical performance are highly warranted.

Management of tendon injuries currently follows 2 routes: conservative (rest, rehabilitation and pain relief by drugs) or surgical and the healing outcome of both strategies is similar. Experimental and clinical studies have shown that mobilization of the patient is more beneficial than immobilization [4]. As to surgery, conventional treatment comprises sewing torn tendons in place; those that pull free of the bone can be re-attached. However, these surgical repairs do not last for a long time – again, novel therapeutic strategies based on biological approaches are needed. In case of extensive damage, grafts (allo- or autografts) have to be used which either bear the risk of infection and rejection (allograft) or donor site morbidity (autograft).

In addition to the difficulties encountered with tendon regeneration per se, the second major obstacle in surgical repair relates to the enthesis i. e. the site of connection between tendon and bone which is difficult to reproduce. And, such a restored tendon-bone attachment site is the weak link. The attachment sites of tendons to bone are called osteotendinous junction (OTJ or enthesis) that occur in two different forms, according to their histological appearance. The specialized structure of OTJs prevents collagen fibre bending, fraying, shearing, and failure [5]. A fibrocartilaginous (or direct) enthesis 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 [6]. The fibrocartilage cells synthesize extracellular matrix that is rich in aggrecan and collagen type II, both of which are typical of articular cartilage. In contrast, a fibrous (or indirect) enthesis lacks the fibrocartilage intermediate zone and is made up of tendon and bone

zones only. In any case, full recovery from injury at the junction takes at least 6 months – long for a conventional patient and even longer for a professional sportsman.

II. Towards future innovative strategies: Animal models.

Despite considerable progress, no optimal solution has been found for the treatment of various sports-related injuries, including muscle injuries, ligament and tendon ruptures, central meniscal tears, cartilage lesions, and delayed bone fracture healing [2]. Therapeutic strategies based on developing novel biological approaches are therefore discussed in the following paragraphs.

Local delivery of growth factors. Growth factors are proteins that stimulate cell division upon binding to their appropriate cell-surface receptor(s). One class of growth factors with special relevance to the musculo-skeletal system is the transforming-growth factor-beta family which includes bone morphogenetic proteins, BMPs. BMPs have an important role during bone formation and regeneration and were discovered due to their bone inducing capacities after implantation at ectopic sites. They are dimeric molecules, most often composed of two identical subunits which transmit their signals predominantly through Smad proteins, a group of related intracellular proteins, from ligand-activated cell surface receptors to the nucleus. Activated R-Smads form heteromeric complexes with the common mediator Smad4 (co-Smad4) that translocate into the nucleus where, in combination with transcription factors, they regulate gene expression. A constitutively active R-Smad – i. e. a Smad protein that is constantly active even in the absence of ligand - can be generated artificially and will be further discussed below.

In general, with growth factor approaches, the major problems are firstly, high doses are needed to be therapeutically efficient which may elicit immune responses, secondly, they easily break down in the body and have very brief half-lives in vivo of only hours whereas a normal healing process takes at least 2 weeks. The use of growth factor proteins to promote healing is severely hindered by the difficulty of ensuring their delivery in minimal yet therapeutically efficient levels to a specific injured site. Various strategies based on scaffolds and biomaterials are therefore being developed

(polymers, pumps, heparin, etc.). Alternatively, surfaces of conventional materials may be altered by chemical or physical processes (functionalization by chemical groups, laser structuring of appropriate materials etc.). For bone repair, approaches including non-specific and non-covalent absorption of BMP2 on surfaces are providing even better results. Another approach is to chemically modify titanium prostheses or other materials, if necessary including reactive groups that are able to bind growth factors and to release them slowly. We and others could show that such an approach is able to induce biological activity in model cell lines and in vivo as well, see, e. g. [7,8]. Despite many successful efforts to solve the problem of long-term delivery by strategies like the one just discussed many researchers may prefer gene-therapeutic strategies (cf. below) or a combination of both, biomaterials and genetically engineered cells or stem cells.

Gene therapeutic strategies.

In order to transfer genetic material into the target cell(s) specific gene delivery systems called vectors are necessary. They include appropriate regulatory genetic elements and the gene of interest. Such vectors can be based on viruses or on plasmids. **Viruses** have elaborate systems for transfer of their genetic material into host cells where they replicate themselves and are finally released to infect new cells. Their properties regarding gene delivery can be separated from the pathological consequences of viral replication by removal of selected viral genes. This not only removes the capacity of unwanted and uncontrolled self-replication but also creates space to insert therapeutic genes. Such replication-deficient viral vectors are produced in special cell lines or by transient transfections that provide the viral genes required for replication and packaging into infectious virus. Since they lack the packaging signals that are only attached to the therapeutic gene the viral genes cannot be packaged into the ensuing virus. Viral vectors have been generated from a wide variety of viruses and therefore, comprise a range of different properties from which they can be selected as applicable. Several systems allow stable and long-term gene expression. **Plasmids** (non-viral vectors) or naked DNA, on the contrary, are non-infectious and exhibit minimal toxicity.

However, they have several drawbacks since their mode of delivery within cationic liposome complexes, as ballistic particles, by calcium phosphate precipitation, or by electroporation is quite unspecific. Transfer efficiency in general is low and expression of the therapeutic gene takes place for only a limited time due to degradation.

Gene transfer can be performed either *in* or *ex vivo*, and both strategies need to be considered dependent on the individual application and the most suitable route of administration. In *ex vivo* gene transfer, cells are genetically modified in culture, selected for successfully modified cells by screening or sorting procedures and then given to the recipient either systemically (mainly the blood) or locally (e. g. intra-articularly). The selection processes make *ex vivo* strategies highly efficient but autologous cell harvesting is an additional burden for the patient and the subsequent cell modifications are time-consuming and expensive. A major advantage but simultaneous limitation of this strategy is that in many cases, the treatment could or should be patient-specific to avoid long-term immunosuppression. Examples of therapeutic animal studies in the soft tissues reviewed here will be discussed below in combination with cellular approaches.

In vivo gene transfer, in contrast, means that a vector with the gene of interest can be prepared for the treatment of many individuals which also reduces the costs for application. In addition, such an approach allows the use of nonintegrating vectors that will lead to relatively short-term gene expression in mitotic (dividing) cells but long-term gene expression in post-mitotic cells. In the present context, this applies specifically to muscle fibres. As a disadvantage, no selection and screening of successfully modified cells is possible. Such a somatic gene therapy is legal and would be sufficient for athletes' needs. Local injection at the injury site seems the method of choice. The major problem is to ensure that the necessary genes are expressed in the right cells.

We will later in this review focus on athletes. Here, the main target of gene therapy (either for enhanced recovery following injury or degeneration or, for improved performance during competitions as "gene doping") is the musculo-skeletal system. Systemic delivery in the musculo-skeletal system faces special hurdles since cartilage and tendon lack sufficient blood supply.

Despite all efforts, gene therapy is almost exclusively experimental medicine, far from routine applications, and much additional preclinical and clinical studies are necessary to prove efficacy and safety [9]. The misuse of this technique for performance enhancement of athletes bears the risk of serious health problems or side effects including death when used in otherwise healthy people without a clinical need.

Cell- and stem-cell derived therapies including genetically modified cells.

Due to the limitations of pure growth factor or gene therapy many labs investigate the usefulness of cells. For regeneration differentiated cells may be helpful as such or by secreting factors (“trophic effects”) that stimulate neighboring or recruited cells to induce a healing response whereas stem cells may be particularly helpful by differentiating themselves into appropriate cell types. Stem cells have elicited broad interest and excitement due to the promise that either embryonic, adult or induced pluripotent stem cells (derived from somatic cells by overexpression of specific factors) could be used for tissue regeneration for injuries where current therapeutic strategies lack efficiency. **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. Alternatively, since **human mesenchymal stem cells (MSCs)** are thought to be non- or only faintly immunogenic even the use of allogenic donor cells might become feasible. They have – due to their possible use in regenerative medicine and tissue engineering – attracted much interest [10]. The transplantation of MSCs into a variety of injured skeletal tissues has been shown to promote healing. In the gene-therapeutic setting MSCs may be intentionally modified by overexpression of growth or transcription factors to more efficiently conduct their differentiation into appropriate cell types or by secreting factors that stimulate neighboring or recruited cells to induce a healing response. Recently, a subpopulation of human perivascular cells was identified that express both pericyte and MSC markers (one hallmark is expression of the surface antigen CD146, melanoma-associated cell adhesion molecule, MCAM) *in*

situ, both *in vivo* and *in vitro*. The isolated population can be expanded and is clonally multipotent in culture [11]. CD146 positive pericytes can also be serially transplanted to create a hematopoietic environment [12]. These cells seem to be superior to the conventionally isolated MSCs since each single clone is multipotential, and they can be kept in culture for at least 17 weeks or 14 passages [11] whereas conventional MSCs go into senescence already around 6 passages. The use of these stem cells may notably enhance human therapeutic strategies for the musculo-skeletal system in the future. Alternatively, the recently described and rapidly expanding field of **induced pluripotent stem cells** holds great promise of creating patient-specific cells for therapy. Several studies have demonstrated their utility in animal models of human disease like sickle-cell anemia [13] whereas studies in the musculo-skeletal system are still missing. However, an inherent tumorigenic potential of these cells has to be closely observed.

As with growth factors, one of the challenges with (stem) cells is to determine the best way to deliver them to their target tissue [14]. To do so, several different biomaterials and scaffolds are in use similar to the ones described for growth factors. One elegant way is to suspend the cells in a viscous fibrin gel which hardens after injection into the body and from which cells can exert their functions. In addition to the mode of delivery, cell transplantation includes more challenges: the way to obtain appropriate cells and to ensure their survival in the recipient.

Exercise and soft tissue regeneration.

The musculo-skeletal system is particularly subject to mechanical stimuli and loading to properly exert its functions; loss of loading rapidly results in loss of functionality. As a consequence, all components of the musculo-skeletal system can be influenced beneficially by exercise and sports and the importance of mechanical stimuli as biological regulators for connective tissue physiology and pathology has been well documented [15-17].

In muscle, exercise may induce satellite cell proliferation and muscle growth [18]. Mechanical stimulation influences metabolic activity, gene expression, and myogenesis as well as fiber

alignment [1]. Likewise, many tendon engineering protocols have relied on the application of mechanical load as reviewed, e. g. in [19] or [4]. Recent attempts to optimize the functional properties of engineered cartilage have focused on using bioreactors to incorporate mechanical loading environments *in vitro* to mimic *in vivo* conditions [1]. In this context, dynamic deformation, hydrostatic pressure, fluid flow, and shear stress were tested, and beneficial effects of mechanical loading on cartilage constructs have been widely reported.

Examples of novel treatment strategies in animal models.

Muscle. Muscle regeneration occurs early in the healing process. It usually begins 3 to 5 days after injury, peaks around the second week, and thereafter declines [18]. Various growth factors like IGF-1 (insulin-like growth factor-1), bFGF (basic fibroblast growth factor), or NGF (nerve growth factor) can improve muscle regeneration during these early stages [18]. Local injection of IGF-1, bFGF, and – although to a lesser extent – NGF after injury increases the number and size of regenerating myofibers in different mouse injury models. Amongst these, IGF-1 has the greatest beneficial effects since it can increase the efficiency of muscle regeneration and muscle strength *in vivo* [20]. The so-called Schwarzenegger mouse which is transgenic for IGF-1 presents with enormous muscle generation i. e. 20 – 30 % greater than in normal mice. In addition, these mice live longer and recover more quickly from injuries [21].

One potential advantage associated with human recombinant growth factors in the treatment of muscle injuries is the ease and safety of the injection procedure that, however, requires a high amount of factors necessary due to rapid clearance by the bloodstream.

The intramuscular implantation of myogenic cells is an approach to develop a therapeutic tool for myopathies, i. e. for diseases with a genetic background [22]. Most studies have been performed on animal models for and patients with Duchenne´s muscular dystrophy. For treatment of skeletal muscle, differentiated muscle cells cannot be used because they can neither be properly manipulated nor implanted. Instead, cellular therapy of muscle relies on satellite cells which are the muscle stem

cells located on the outer side of muscle fibers, between the basal lamina and plasma membrane.

After injury, these cells are triggered to divide and to recapitulate the myogenic events: formation of myoblasts, myocytes, and finally of multinucleated myotubes and muscle fibers. Satellite cells are easily amenable to therapeutic manipulations since they can readily be isolated from muscle biopsies and maintained in culture.

The main factor during intramuscular cell injections to consider is the fact that injected cells fuse mainly with the myofibers damaged by the injection trajectories. As a consequence, cell implantation protocols should include “high density” injections: 100 cell injections/cm² may serve as a guideline [22] but may entail different risks like developing a compartment syndrome in muscles that are enclosed in a rigid osteofacial space. To ensure the long-term survival of myoblast allografts [22], an appropriate immunosuppression would be required. As a consequence, like in the case of gene therapy, such strategies should currently be limited to patients suffering from severe muscular diseases but should not be applied to athletes to speed up a healing process.

The potential therapeutic value of MSCs for muscle repair has been a controversial issue. However, recent studies show that GFP-labeled human MSCs were able to undergo myogenesis upon transplantation. Histology revealed mature muscle characteristics in most GFP-positive myofibers. In addition, some of the cells even seemed to become Pax7 expressing satellite cells [23]. Most recently, the human perivascular cells expressing both pericyte and MSC markers including CD146 have convincingly demonstrated a high potential for muscle fibre generation. This was true not only for cells isolated from skeletal muscle but also for perivascular cells obtained from other donor sites [11].

Readers interested in learning more about therapeutic strategies for muscle injuries might want to see the excellent review by Huard et al. [2].

Cartilage. In contrast to other tissues of the musculo-skeletal system, therapeutic approaches using growth factors alone (i. e. in the absence of cells) are not often investigated since they have been even less promising than strategies involving cells. In principle, fully differentiated chondrocytes

would be the ideal cell candidate for cartilage repair. Autologous chondrocytes derived from a minor load-bearing area and injected under a periosteal flap can repair full-thickness chondral defects. This procedure (trade name: Carticel™, Genzyme Biosurgery) is the only FDA-approved cell-based therapy for cartilage repair [1]. Apart from being costly and non-conclusively superior to other methods this autologous technique suffers from potential donor side morbidity, limited availability of chondrocytes, and dedifferentiation of the isolated chondrocytes in monolayer culture. MSCs as a potential alternative to chondrocytes have not proven to be very successful yet. Specifically, their potential to differentiate into chondrocytes in response to transforming growth factor-beta is quite limited as a panel of studies have shown.

In the menisci, there are special cells with fibroblast- plus chondrocyte-like properties called fibrochondrocytes. Recent evidence has pointed to the potential use of MSCs for meniscal regeneration: GFP-labeled rat MSCs were seeded in fibrin glue and used to treat meniscal defects. These cells were detectable until 8 weeks after transplantation and promoted repair [24]. However, for a successful meniscal therapy, further optimization of regenerative conditions and factors is necessary. Yet another challenge resides in the fact that a functional meniscal tissue must exhibit notable anisotropic mechanical properties resulting from a highly oriented underlying extracellular matrix [1]. In order to generate a scaffold which has controllable, anisotropic properties and mimicks meniscal extracellular matrix fiber alignment to direct fibrochondrocyte orientation electrospinning technology was used [25]. This technique is based on a biodegradable polymer and results in cartilage with about 20 % of the strength of native cartilage but with quite small pores that are difficult for chondrocytes to penetrate [3].

A variety of other natural and synthetic biomaterials have also been investigated. Guilak et al. [26] now have created a scaffold which leaves more space for the cells than the electrospinning material. In particular, a different biodegradable material was used in a novel three-dimensional weaving technique which gives compressive, tensile, and shear strength highly similar to native cartilage but degraded too rapidly in vivo. Using another polymer as basis, the group hopes to go into clinical

studies in 2010 [3]. Chiang et al. [27] seeded chondrocytes into gelatin microbeads mixed with a collagen type I gel. This enhanced repair in a porcine cartilage defect model, and the chondrocyte phenotype was maintained better than without the carrier. Many more scaffolds and matrices have been under examination (reviewed, e. g., in [1], [28]) but so far showed variable effects on cell behaviour and function.

Viral vectors have been used in several studies to deliver therapeutic genes to chondrocytes in vitro [2]. In addition, these modified cells were applied in collagen gel or organ tissue explants. In vitro delivery of genes for BMP2, IGF-1, and TGF-beta to articular chondrocytes has been shown to increase proteoglycan synthesis by the cultured cells (see, e. g., [29,30]). After retroviral overexpression of BMP-7 in mesenchymal stem cells and cell delivery on a polyglycolic acid scaffold a rabbit osteochondral articular cartilage defect showed improved healing [31].

In conclusion, there is still a long way to go and develop the optimal strategy and cell source for cartilage repair especially with respect to functional and biomechanical behaviour.

Tendon. Within the past years the American FDA (Food and Drug Administration) has approved BMP2 and BMP7 for human application, mainly for bone repair. However, BMPs may also prove efficient for cartilage and tendons. BMP12, e. g., was tested for healing of rotator cuff injuries in sheep [32] using two modes of long-term delivery: a paste and a biodegradable collagen sponge to release BMP12. In treated sheep, shoulders were three times stronger (1.8 kN as compared to 0.6 kN) than in sham-operated animals. However, this strength was much less than for a normal, non-ruptured shoulder: 3.7 kN.

As mentioned in the introduction, one major obstacle in tendon ruptures is the fact that tendon-bone junctions cannot easily be reconstructed to provide secure healing: Especially fibrocartilaginous osteotendinous junctions do not regenerate properly and all attempts for generation of such an enthesis with four zones have failed so far and resulted in fibrous enthesis only [33]. And, an enthesis in the wrong anatomic location entails limited mechanical strength. Therefore one recent study investigated the effect of rhBMP-2 as compared to noggin, a potent BMP inhibitor. Indeed,

BMP2 demonstrated a strong, positive dose-dependent effect on osteointegration at the tendon-bone junction. In contrast, noggin decreased osteointegration. Further studies are necessary to investigate the potential clinical application of enhancing healing and decreasing recovery time with bone morphogenetic proteins [34]. A recent study by Hashimoto et al. [35] used application of BMP2 on native rabbit tendon which resulted in ossicle formation including a cartilage region but such an approach requires two surgeries, one to ossify a graft tendon, and a second one to reconstruct the injured site.

Delivery of therapeutic genes has also been investigated for its beneficial use in de novo tendon formation and tendon repair. Several studies were conducted with reporter genes based mainly on adenoviruses in order to assess the overall feasibility of gene transfer into tendon tissues. These studies reveal that direct administration of adenoviruses is possible but may not be the optimal choice for tendon repair since their dense extracellular matrix apparently interferes with efficient infection [10].

In principle, the primary sources of cells for tendon regeneration are currently expected to be tenocytes, MSCs, or tendon stem cells. Tenocytes, derived from tendon progenitor cells, secrete large amounts of collagens which aggregate into basic tendon fibrils. Two major problems with the tenocyte as a therapeutic vehicle, however, arise: Firstly, tendons contain relatively low amounts of cells resulting in limited availability, secondly, they do not proliferate well in culture, dedifferentiate, and lose the characteristic tenocyte morphology [36].

Several studies using MSCs have been conducted and strategies for a successful MSC-dependent repair of tendon tissue have been described. Repair of the Achilles, rotator cuff and patellar tendon have been reported. De novo tendon-like tissue formation was observed after three months [37]. In another study MSC-collagen composites were used for tendon repair of the patellar tendon. In this study, a higher capacity to resist maximum stress than natural repair was reported [38].

Our group could show that a murine MSC-like cell line genetically engineered to express BMP2 and a biologically active Smad8 variant differentiates into tendon-like cells. The engineered cells

demonstrated the morphological characteristics and gene expression profile of tendon cells both *in vitro* and *in vivo*. In addition, following implantation into an Achilles tendon partial defect, the engineered cells were capable of inducing tendon regeneration [39]. More recently, we could show that by appropriate manipulation of BMP2 and constitutively active Smad8 expression levels the formation of either pure tendon-like tissue or, alternatively, tendon attached to osteotendinous junctions, is possible. Interestingly, C3H10T^{1/2} cells generate fibrocartilaginous entheses whereas human MSCs give fibrous entheses (Shahab-Osterloh et al., unpublished). Especially the creation of fibrocartilaginous entheses *in vitro* would be therapeutically highly desirable.

In 2007, researchers reported that they successfully isolated small numbers of cells with stem cell characteristics from the extracellular matrix of both mouse and human tendons – so called **tendon stem cells**. Their study showed that adult tendons contain a rare subset of stem cells that can be isolated, grown in the lab, and then used to generate tendon-like tissues at least in mice [40]. Future studies will have to show whether tendon stem cells may be useful in human therapies – one question to answer will be whether these cells can be made available in high enough numbers in a reasonable period of time.

III. Examples of therapeutic strategies in athletes.

Sports injuries usually involve tissues that display a limited capacity for healing. Experience is still limited in human athletes due to concerns about doping (to be discussed by H.H. Haisma et al., chapter 12, this book). However, in the treatment of athletic injuries growth factor, gene and or cell therapies may induce more rapid healing or less side effects from the repair process, such as adhesions and fibrosis. Studies in this field are limited and there is no clear evidence supporting long-term enhanced athletic performance arising from such therapies in animal studies [41]. For instance, expression of growth and differentiation factor-5 (GDF-5) in the region of a joint-associated tendon repair improved the range of movement but did not change the - limited - biomechanical properties in a mouse model [42].

Tendon. The Anterior Cruciate Ligament (ACL) consists of a tough band of tissue that connects the thighbone to the shinbone. It stabilizes the knee and enables its motions, including not only stretching but also turning. A torn ACL does not heal well even if surgeons manage to sew the ends back together which means that athletes cannot go for competition for several months – and especially for a professional athlete, this is highly unpleasant situation. Conventional therapy means that often the patellar or hamstring (i. e. semitendinosous plus gracilis) tendon of the patient is used as an autograft to replace the torn ACL. This, however, entails significant donor site morbidity and often proves to be even more disabling than the original defect. Grafting of the central third of the patellar tendon-bone may also lead to patella baja, arthrofibrosis, adhesion to the fat pad, and patellofemoral pain which is hardly helpful for normal patients and even less for sportsmen. Allografts have seen limited use except in revision surgery or for multiple ligamentous injuries [4]. Many studies have been conducted in animals. In particular, there are many similarities between the weight-bearing tendons of the horse and those of the human athlete (e. g. Achilles tendon) in their hierarchical structure and matrix organization, their function and the nature of the injuries sustained. The horse is to be considered as a very special and precious animal athlete suffering often from strain-induced tendon or ligament injuries, resembling consequences of athletic endeavour in humans. Both in horses and in humans, this results in high morbidity and often prevents return to the original performance. Many veterinarians, therefore, are considering therapeutic strategies based on MSCs which, similar to humans, can be preferentially harvested either from equine bone marrow or adipose tissue for treatment of tendon ailments. For horses, MSCs have been applied for the surgical treatment of subchondral bone cysts, bone-fracture repair, and cartilage repair [43], and prominently, for tendon injuries. These studies at the same time not only help to cure the horses but also advance our understanding of orthopaedic treatment options and enhance translational research into the clinics.

Muscle. Depending on the type of sport, muscle injuries constitute between 10 and 55 % of all injuries sustained by athletes [2]. Of relevance for muscle-targeted therapies and gene doping,

promoters derived from muscle-creatinine-kinase, smooth muscle-cell α -actin and myosin-heavy and light chain have been used in cell culture and animal model studies to achieve muscle-specific expression of recombinant proteins. At present such strategies should be applied only to patients suffering from severe muscular diseases but not to athletes to speed up a healing process because there are many more risks than potential benefits.

Cartilage. As alluded to in section II, the current state of research for cartilage engineering of human patients is not yet entirely promising. However, improvements in culture conditions and scaffold materials, incorporation of bioactive factors, and recognition of the role of mechanical stimulation in cartilage formation and more novel techniques are constantly developed to improve not only the biological behaviour but also the mechanical properties [28]. The 20 % of the strength of the native cartilage that have been obtained by several groups are still far from the clinical needs of patients. Such innovative strategies will certainly be of benefit for athletes under the above-mentioned precaution that they will not be abused for doping and give them an illegal advantage over their non-treated competitors.

Acknowledgements.

The authors gratefully acknowledge the support by the EU integrated project “GENOSTEM” and by the SFB 599 of the German Research Foundation (DFG) in the past and present years. We apologize to all of our colleagues whose work we could not cite due to space constraints.

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