Chapter 1

Novel Therapies for the Management of Sports Injuries

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1. Introduction

With the contemporary active lifestyle and widespread professionalism in sport, the need for high-end injury therapies is growing. Conservative principles in managing various sports injuries usually do not meet the need of athletes and their coaches. In order to achieve better and faster recovery after injuries, significant effort has been made in the recent decade among researchers. Local growth factor application, targeted therapies using recombinant proteins and tissue engineering represent promising groups of future therapeutic options with promising results.

Healthy tendons and ligaments get injured either by a single application of force or by a repeated or sustained action that alters their mechanical characteristics. Genetic disorders, aging, decreased vascularity, endocrine influences, nutritional status, inactivity, immobilization, and exercise may cause tendon degeneration, thus rendering the tendon or ligament more susceptible to injury when force is applied. Hypovascularity is hypothesized to play the major role in this degeneration, both directly by causing an ischaemic environment for the fibroblast and indirectly both by contributing to the production of free radicals and by allowing for tissue hyperthermia to occur. Conservative management, such as rest, corticosteroid injection, orthotics, ultrasound, laser treatment, or shockwave treatment provide pain relief but, when they fail, surgery is required. Local growth factor application and tissue-engineering strategies, such as the development of scaffold microenvironments, responding cells, and signalling biofactors are currently generating potential areas for additional prospective investigation in tendon or ligament regeneration.

Cartilage tissue also comprises of limited intrinsic potential for healing due to the lack of blood supply and subsequent incomplete repair by local chondrocytes with inferior fibrocartilage formation. Surgical intervention is often the only option, but the repair of damaged

cartilage is often less than satisfactory, and rarely restores full function or returns the tissue to its native normal state. The new concept of cartilage tissue preservation uses tissue engineering technologies, combining new biomaterials as a scaffold, applying growth factors and using stem cells and mechanical stimulation.

Skeletal muscle, on the other hand, has a great regenerative capacity; however, this process if often incomplete because of partial fibrous scar formation. Conservative therapies including cryotherapy, resting, physical therapy and pain relief medications don't often give satisfactory results and can even be controversial. While surgery is reserved for bigger tissue defects, the need for antifibrotic agents to improve muscle repair after injury is obvious. These and platelet-derived growth factors represent the future of biological therapies for this common type of sports injury.

Novel tissue-specific therapies are mainly molecular, based on pathophysiological processes after injury. Although they seem to significantly accelerate healing and shorten the recovery time, their true goal is to achieve better and more functional repair.

2. Biological therapy for better muscle regeneration

While spontaneous muscle fibre regeneration usually occurs after muscle injury, this process can often be slow and incomplete and accompanied by fibrotic infiltration, which compromises the restoration of contractile function [1]. Scar formation is the result of excessive wound healing leading to a poor functional outcome after trauma and surgery [2]. Successful muscle repair after injury is important for restoring mobility and patients' quality of life. We therefore have an important medical need for drugs that can promote or hasten muscle fibre regeneration, reduce fibrosis, and enhance muscle function [1].

Despite the clinical significance of muscle injuries, current treatment principles for injured skeletal muscle lack a firm scientific basis, and are based on performing RICE (Rest, Ice, Compression, Elevation) and sometimes prescribing non-steroidal anti-inflammatory drugs (NSAIDs). However, increasing evidence indicates that the administration of NSAIDs decreases regeneration and increases fibrosis by inhibiting inflammation [3, 4].

Incomplete muscle fibre regeneration and fibrotic infiltration can lead to long-term functional deficits and physical incapacitation [1]. In recent years of muscle regeneration research, many agents have been described to have a significant antifibrotic effect in patients with heart or kidney disease and systemic sclerosis. Consequently, researchers are testing these for muscle healing, as therapeutic targets are the same. Although these agents play a life-saving role in the previously mentioned diseases, their importance for muscle injuries could be substantial and for athletes specifically, vital.

Transforming growth factor-Beta (TGF-β) and myostatin have been identified as the main factors that stimulate fibrotic differentiation. It has been shown in *In vitro* and *In vivo* studies that drugs with anti-fibrotic properties that can prevent or minimize scar formation have the potential of standalone or adjuvant therapies (Table 1). Although the World Anti-Doping

Agency (WADA) prohibits any use of myostatin inhibitors in athletes, their potential to act only therapeutically at the site of injury without any performance enhancement may play an important role in muscle injury therapy in the future. In the following chapters, various agents are described that reportedly have beneficial effects on muscle healing after injury.

Class	Agent
Recombinant proteins	Follistatin
	Decorin
	Interferon-γ
	Suramin
	Relaxin
Autologous growth factors	Platelet-rich plasma (PrP)
Other bioactive agents	Mannose-6-phosphate (M6P)
	N-acetylcysteine (NAC)
	Angiotensin receptor blockers (ARBs)

Table 1. Agents with proven anti-fibrotic effects in *In vitro* or *In vivo* studies of skeletal muscle regeneration after injury

2.1. Recombinant proteins in muscle regeneration

Follistatin is an autocrine glycoprotein expressed in nearly all tissues of higher animals, with multiple effects on skeletal muscles as well as other tissues [5]. It is a functional antagonist of several members of the TGF-β family of secreted signalling factors, including myostatin - the most powerful inhibitor of muscle growth characterized to date [6, 7]. Follistatin was previously known as FSH-suppressing protein (FSP) as it was found to have inhibitory effect on pituitary secretion of follicle-stimulating hormone (FSH) [8]. Research into the development of therapies to antagonize myostatin has led to the discovery of several new functions exhibited by follistatin [9]. Several *In vivo* studies on follistatin have shown that it directly inhibits myostatin and also reduces myostatin-induced muscle wasting after systemic administration [5, 10]. In recent research, Zhu et al. reported on the stimulative effect of follistatin on MyoD, MyF5, and myogenin expression, which are myogenic transcription factors that promote muscle differentiation. They also showed its inhibitory effect on myostatin, activin A, and TGF-β, all of which are negative regulators of muscle cell differentiation [9]. Although various myostatin inhibitors have been described, follistatin can modulate other regulators of muscle mass in addition to myostatin [11]. For example, follistatin administration to MSTN-1- mice caused muscle mass increases beyond that stimulated by myostatin depletion [12, 13], suggesting that this may be a more potent approach than targeting myostatin alone. Intramuscular administration of gene therapy vectors expressing follistatin has increased muscle mass and strength in both young and aged mdx mice, as well as in nonhuman primates [14]. In order to investigate the mechanisms of the follistatin-induced muscle hypertrophy, Gilson et al. used irradiation to destroy the proliferative capacity of satellite cells. They found that not only inhibition of MSTN, but also of activin (ACT) and

proliferation of satellite cells are involved in follistatin-induced muscle hypertrophy [12]. Follistatin thus might offer a novel therapeutic strategy for muscular injuries and dystrophy by suppressing the progression of muscle degeneration and permitting skeletal muscle mass restoration [15]. However, before translating follistatin-based therapies from the bench to the bedside, clear mechanisms of how follistatin promotes muscle regeneration require extensive investigation.

Decorin is a component of the extracellular matrix in all collagen-containing tissues [16] and is expressed at high levels in skeletal muscle during early development [17]. It is a small leucine-rich proteoglycan that can modulate the bioactivity of growth factors and act as a direct signalling molecule to various cells [18]. It was found to neutralize the effects of myostatin in both fibroblasts and myoblasts. It has been implicated in cell proliferation and differentiation due to its ability to bind growth factors and has been found to interact with collagen, fibronectin and thrombospondin, hence influencing processes such as fibrillogenesis, cell adhesion, and migration [19]. Fukushima et al. proved that decorin also inactivates the stimulating effect of TGF-β in myofibroblasts *In vitro*, which has a beneficial effect in muscle fibrosis and leads to enhancement of muscle regeneration and strength [20]. Recent reports showed that the injection of decorin into lacerated muscle improves both muscle structure and function, enabling nearly complete recovery of muscle strength [20-22]. Besides reducing fibrosis, decorin promotes muscle cell differentiation by upregulating follistatin, PGC-1α, and myogenic genes, including MyoD [23]. Thus, decorin appears to be a new molecule in the myostatin-signalling pathway [24-26]. It has been reported that muscle cells produce decorin and myostatin proteins at the same time, and that prenatal and postnatal expression of myostatin is similar [17].

Interferon (IFN)-γ is an inflammatory cytokine that was first identified as an antiviral factor [27]. A primary function of IFN-γ is activation of macrophages through the classical pathway, which promotes pathogen killing [28]. Later, IFN-γ was recognized as a pleiotropic cytokine that also plays a role in regulating different immune responses as well as influencing many physiological processes [29]. It has also been shown by Foster et al. in 2003 to not only down-regulate endogenous collagen expression, but also to effectively block TGFβ -mediated increases in collagen protein levels [30]. INF-y also inhibits TGFβ signalling by inducing expression of SMAD 7, which participates in a negative feedback loop in the TGFβ signal transduction pathway [31]. Therefore, IFN-γ is thought to be an antifibrotic factor during tissue repair that can reduce synthesis of the extracellular matrix by disrupting signalling by the profibrotic cytokine TGF-β [31, 32]. IFN-γ has been found to influence skeletal muscle homeostasis and repair. In 1999 Shelton et al. reported age-dependent necrotizing myopathy in a transgenic mouse that constitutively overexpresses IFN-γ at the neuromuscular junction [33]. On the other hand, the administration of IFN-γ appears to improve the healing of skeletal muscle, limit fibrosis and therefore limit the function of a regenerating muscle [30]. In 2008, Cheng et al. showed that IFN-γ is expressed at both mRNA and protein levels in skeletal muscle following injury, and that the time course of IFN-γ expression correlated with the accumulation of macrophages, T-cells, and natural killer cells, as well as myoblasts, in damaged muscle. The admin-

istration of an IFN-γ receptor-blocking antibody to wild-type mice impaired induction of interferon response factor-1, reduced cell proliferation and decreased the formation of regenerating fibres [29]. In 2008, Chen et al. reported a synergistic effect of IFN-γ and IGF-1, which is known to have beneficial effects on muscle regeneration after injury. They showed that IFN-γ injected into injured muscle has the effect of anti-fibrosis, which is more significant than that of IGF-1. They concluded that a combined injection could improve muscle regeneration, while inhibiting fibrosis simultaneously, and promote the healing of injured muscle [34]. *Suramin*, an antiparasitic and antitumor drug, acts as a TGF-β inhibitor by competitively binding to the growth factor's receptor [35, 36]. It has been evaluated for potential clinical applications and has shown antifibrotic effects in chronic kidney diseases, wound healing of rabbit conjunctiva and glaucoma after trabeculotomy [37, 38]. Chan et al. showed that suramin effectively inhibits fibroblast proliferation and neutralizes the stimulating effect of TGF-β on the proliferation of fibroblasts *In vitro*. *In vivo*, they showed that the injection of suramin two weeks after strain injury reduces muscle fibrosis and enhances muscle regeneration, and thereby leads to improved muscle strength recovery [39]. Although suramin can lead to side effects when administered intravenously, local intramuscular injection may not elicit the same deleterious effects and could be very useful in improving muscle healing [39]. Taniguti et al. evaluated the effect of suramin on fibrosis in mdx mice, where TGF-β is highly unregulated in the diaphragm and the quadriceps muscle. Mice received suramin for seven weeks while performing exercise on a treadmill to worsen disease progression. Suramin protected limb muscles against damage and reduced the exercise-induced loss of strength over time. These findings support the role of TGF-β in fibrogenesis and myonecrosis during the later stages of disease in mdx mice [40].

Relaxin, a polypeptide cytokine/growth factor, is a member of the insulin-like growth factor (IGF) family. The historical role of relaxin has been in reproduction, in which it functions to inhibit uterine contraction and induce growth and softening of the cervix. It can reduce type I and type III collagen deposition, increase procollagenase synthesis, and, by doing so, reduce fibrous scar tissue formation in many tissues [41, 42]. In an *In vivo* model of pulmonary fibrosis, relaxin treatment dramatically decreased bleomycin-induced collagen content in the lung, alveolar thickening, and improved the overall fibrosis score [43]. Recent studies using the relaxin-null mouse model have demonstrated age-associated pulmonary fibrosis in these animals that can be reversed by relaxin treatment [42]. In an innovative study, adenoviral-mediated delivery of relaxin was used to treat cardiac fibrosis caused by transgenic overexpression of the β2-adrenergic receptor, resulting in a dramatic decrease in interstitial collagen content in the left ventricle, but not other (nonfibrotic) chambers of the heart [44]. In a recent study, Mu et al. injected relaxin intramuscularly into the injured site of the mouse to observe its function *In vivo*. Results showed that relaxin promoted myogenic differentiation, migration, and activation of matrix metalloproteinases (MMPs) of cultured myoblasts *In vitro*. Relaxin also promoted activation of muscle satellite cells and increased its local population compared with non-treated control muscles. Meanwhile, both angiogenesis and revascularization were increased, while the extended inflammatory reaction was repressed in the relaxin-treated injured muscle. [45]

2.2. Platelet-rich plasma in muscle injury therapy

Among new therapeutic options for achieving more efficient healing, autologous thrombocytes have a very important place. Although there is no randomized prospective study confirming its value, platelet-rich plasma as a source of autologous growth factors is thought to be used by many sports physicians for treating muscle injuries. The use of platelet-derived preparations was prohibited by WADA until 2011 but was removed from the list after considering the lack of current evidence concerning the use of the method for the purposes of performance enhancement as current studies did not reveal a potential for performance enhancement beyond the therapeutic effect [46].

PrP (or platelet-rich plasma) may be defined as a volume of the plasma fraction of autologous blood having a platelet concentration above the baseline [47]. Normal platelet counts in blood range from 150000/μL to 350000/μL. Platelet-rich plasma contains a 3 to 5-fold increase in growth factor (GF) concentrations, sometimes more [47,48]. Platelet-rich plasma can only be made from anticoagulated blood [47]. The process begins by adding citrate to whole blood to bind the ionized calcium and inhibit the clotting cascade, followed by one or two centrifugation steps to separate red and white blood cells from platelets. When using anticoagulated PrP, activation is critical, as clotting results in the release of GF from the platelet α-granules (degranulation). PrP may be activated immediately before application, or it can occur *In vivo*. There is no consensus on the timing of PrP activation, or even whether activation is necessary at all [47]. Approximately 70% of the stored GF is released within 10 minutes, and more than 95% of the GF is released within 1 hour. Some GF is produced by the platelets during the next 8 to 10 days. Originally, bovine thrombin was used as an activating agent; however, a rare but major risk of coagulopathy from antibody formation has restricted its use for activation. Calcium chloride ($CaCl_2$) and autologous prepared thrombin are now used for activation instead. The $CaCl_2$ is added during the second centrifugation step to form a dense fibrin matrix in which platelets are trapped and release GF. Soluble collagen type I may also be used for activation. It is important to note that the composition of commercially derived PrP products differ qualitatively and quantitatively [49]. The most important difference is in the concentration of platelets as well as in the concentration of leukocytes in the preparation. Whether leukocytes have a positive or negative role is not clear yet. The paradigm suggests that neutrophils infiltrate injured tissue and in the process of assisting the removal of disrupted tissue, exacerbate or increase the original damage [49].

Platelet-rich plasma can potentially enhance healing by the delivery of various GF and cytokines from the α-granules contained in platelets. Platelets also contain subpopulations of α-granules that undergo differential release during activation, a potentially important point in understanding how PrP is activated and acts [47, 48]. Platelets contain, synthesize and release large amounts of biologically active proteins that promote tissue regeneration. Researchers have identified more than 1100 types of proteins inside platelets or on their surface [47-49]. The most commonly studied platelet proteins include platelet-derived growth factor (PDGF), transforming growth factor (TGF-β), platelet-derived epidermal growth factor (PD-EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor I and II (IGF-I, IGF-II), fibroblastic growth factor (FGF), and cytokines, including proteins

such as platelet factor 4 (PF4) and CD40L. The roles of the above listed growth factors are listed in Table 2 [47, 48, 50].

Growth Factor	Target tissue/cell	Function
FGF	Blood vessels, smooth muscle, skin fibroblasts and other cells	Proliferative and angiogenic action Stimulates collagen production
VEGF	Blood vessels	Stimulates vascularisation by stimulating vascular endothelial cells
TGF-β	Blood vessels, skin cells, fibroblasts, monocytes	Stimulates fibroblast production Stimulates production of collagen type-I and fibronectin
PDGF A+B	Fibroblasts, smooth muscle cells, chondrocytes, osteoblasts, mesenchymal stem cells	One of the first growth factors to be expressed after injury
		Stimulates other growth factor secretion Stimulates angiogenesis and macrophage activation
		Chemotaxic and proliferative action on fibroblasts, stimulates collagen synthesis
PD-EGF	Blood vessel, skin cells fibroblasts and other cells	Stimulates epidermal regeneration and wound healing by stimulating keratinocytes and dermal fibroblasts Promotes cell growth, recruitment, differentiation
		Stimulates cytokine secretion
IGF-I, II	Bone, blood vessel, skin, other tissue fibroblasts	Chemotactic for fibroblasts
		Stimulates protein synthesis
		Enhances bone formation
PF-4	Neutrophils, fibroblasts	Stimulates influx of neutrophils Chemotactic for fibroblasts
Myostatin,		Mainly function on bone/skeletal muscle adaptation and repair
Leukaemia inhibitory factor (LIF),		
mechano growth factor (MGF), Bone morphogenetic protein (BMP)		
(a member of TGF-β superfamily)		

Table 2. Growth factors released from platelets and their function

In vitro studies showed that the application of PrP enhances gene expression of the ECM proteins, has mitogenic activity, promotes tenocyte proliferation and induces secretion of other growth factors [47]. Importantly, animal studies showed that all positive effects of PrP

are neglected when no mechanical stimuli are applied to the tendon during the healing period, e.g. if the tendon is immobilised. Besides increased expression of growth factors, PrP was found to increase the expression of matrix degrading enzymes. PrP may also promote antibacterial effects. In addition to opsonophagocytosis, chemotaxis, and oxidative microbicidal activity, platelets and leukocytes can release a variety of small cationic peptides (antibacterial peptides) that have bactericidal activity [47].

When treating ligament injuries with PrP, animal studies suggest that use of PDGF-BB may improve the quality of healing medial collateral ligaments, and in a similar way PrP may influence the healing of other ligaments [51]. The effect of PrP may be dose and time-related [52]. However, extra-articular ligaments showed better wound site filling and increased the presence of finbrinogen and GF when healing as compared to intra-articular ligaments (like ACL), but the application of PRP can improve the results after ACL injury [53].

To date, no major adverse effects of PrP have been noted in humans. No adverse effects were observed when PrP was infiltrated in 808 patients, mainly with osteoarthritis [54]. The use of bovine thrombin for activation may cause a hypersensitivity reaction and is therefore avoided in modern preparation techniques [47]. To date, there is no evidence of a systemic effect of local PrP injection or carcinogenesis. The latter may be mainly due to the short *In vivo* half-lives and local bioavailability of GF produced by PrP [47]. At the moment, PrP is permitted by WADA (The World Anti-Doping Agency) by all routes of administration since 2011 [47].

The International Olympic Committee Consensus Statement expresses that current evidence suggests the use of PrP to be safe. They proposed that what type of PrP product is used and how it has been prepared, validated and tested should be made clear [47].

Suggested techniques for the application of PrP and post-injection recommendations of the International Olympic Committee Consensus Statement are [47]:

1. PrP is considered to act best when placed at the site of injured tissue; therefore ultrasound guidance is advisable for accurate needle placement to the injured site.
2. With respect to tendon administration, there is no agreement on whether the needle should be placed inside the tendon or in the surrounding tendon sheath. In the presence of exudates around the tendon, it is suggested that it be evacuated before PrP is injected.
3. If PrP is administered at arthroscopy, it is suggested that the injection be administered after emptying the joint of arthroscopic fluid. In the case of open surgery, the application of PrP can be undertaken using one of the gel or semi-solid forms.
4. Patients should follow general recommendations after an injection with rest, ice, and limb elevation for 48 hours. Depending on the site of treatment and extent and duration of the condition, patients may follow an accelerated rehabilitation protocol under appropriate supervision.

In the XX *(number needed for chapter: physiology of sports injuries)* chapter of this book, all phases of healing in an injured tissue are described. They are influenced by a number of growth

factors that control cell functions through direct interactions with extracellular parts of the transmembrane receptors. Because thrombocytes represent a major source of growth factors in blood clots, the idea to concentrate them at the site of injury is well accepted. The effects of several individual growth factors have been studied in muscle regeneration. Results from *In vitro* studies are variable; however, their obvious role in regeneration is not to be neglected. Growth factors together with macrophages and products of the cyclooxygenase 2 (COX-2) regulatory pathway regulate the inflammatory phase during regeneration in skeletal muscle [55]. IGF-1 and FGF have been shown to have positive effects on healing and fast-twitch tetanic strength in a murine model of muscle laceration [56]. In case of gastrocnemius contusion, the local application of both GFs lead to higher satellite cell activation in bigger muscle fibre development [57]. Despite the promising potential of PrP for treatment of muscle injuries, some doubts have arisen concerning their use. Due to application of exogenous TGF-β into the tissue, which has been proven to be highly responsible for tissue scarring, some experts are not defenders of this particular therapeutic option. However, in a recent *In vitro* study using human myoblast cell lines it was shown that PrP-derived growth factors promote satellite and muscle cell proliferation as well as inhibiting fibrotic differentiation, mainly due to down-expression of TGF-β [58].

To date there are no randomized control studies confirming the real role of PrP in treating muscle injuries [59], nor was any sample in clinical studies large enough to represent relevant statistical data [48]. However, the preclinical data seems to be promising enough for clinical studies to take place.

2.3. Other bioactive agents to improve muscle healing

Manose-6-Phosphate (M6P) is a natural inhibitor of TGF-β, a carbohydrate molecule with structural similarity to TGF-β and has been shown to reduce its activity [2, 60]. In an experiment by Roberts et al., M6P reduced scarring in incision wounds in rats [61]. In another recent study M6P significantly reduced TGF-β1-mediated transformation of human corneal fibroblasts into myofibroblasts and is therefore a potential modulator of corneal wound healing that may reduce haze after refractive surgery [62]. Regarding the musculoskeletal system, only a few studies on tendons had been performed to date. In an experiment by Bates et al. the antifibrotic effect of M6P was under observation *In vitro* and *In vivo*, where primary cell cultures from the rabbit flexor tendon sheath, epitenon, and endotenon were established and supplemented with TGF-β along with increasing doses of M6P. They also transected and immediately repaired rabbit flexor tendons. M6P solution was added to the repair sites and compared to a placebo group. They found that M6P is effective in reducing TGF-β upregulated collagen production, which correlated with the finding that a single intraoperative dose of M6P improved the postoperative range of motion. Because of its nonimmunogenic property and because it is easily produced, M6P could be an ideal candidate for clinical application in muscle injuries [2]. Yang et al. studied the effects of M6P on TGF-β peptide and receptor expression in order to provide the experimental basis for preventing tendon-healing adhesion by M6P. They found that M6P can significantly decrease the expressions of TGF-β peptide, TGF-β

receptor, TGF-β mRNA and may therefore provide a means of modulating the effects of TGF-β on adhesion formation in flexor tendon wound healing [63].

Although *N-acetylcysteine (NAC)* is a non-toxic aminothiol widely known as an antidote to acetaminophen overdose, it has multiple other uses supported by varying levels of evidence, like chronic obstructive pulmonary disease exacerbation, prevention of contrast-induced kidney damage during imaging procedures, and treatment of infertility in patients with clomiphene-resistant polycystic ovary syndrome [64]. Recent studies have emphasized the role of oxidative stress as the molecular basis of lung fibrosis. NAC has a strong reductive capacity that inhibits the TGF-β-stimulated collagen production in cultured fibroblasts [65]. Moreover, it has been shown by Hagiwara et al. that the aerosolized administration of NAC attenuates the lung fibrosis induced by bleomycin in mice [66] suggesting the suppressing effects of antioxidant TGF-b1 signalling *In vitro* and *In vivo*. A recent study demonstrated that NAC reduces the disulfide bonds of TGF-b1 and changes the bioactive form to the inactive form [67]. It also changes the binding activity of TGF-b1 to its receptor in hepatic stellate cells, suggesting that the effect of antioxidant NAC is based on a direct blockade of TGF-b1 function and signalling. However, whether NAC can modulate the TGF-b1-induced tissue repair, mediator production, and differentiation in human lung fibroblasts has not been fully elucidated [68]. Sugiura et al. recently reported that NAC affects the production of fibronectin and vascular endothelial growth factor (VEGF), which are believed to be important mediators of repair and remodelling. The effect of NAC on the TGF-β induces differentiation to myofibroblasts by assessing a smooth muscle actin (a-SMA) expression [68]. Although treatment with NAC has been shown to attenuate interstitial fibrosis in mouse models of hypertrophic cardiomyopathy mutation and several other pathological states [69], no studies have been performed on the effects of NAC in muscle regeneration after injury. However, since it has been shown to have beneficial effects on diseases that share the same pathophysiological core with the process of muscle fibrosis, the effects of NAC could potentially be beneficial in this pathology as well. Because it is a safe, inexpensive, and well-tolerated antioxidant with a well-defined mechanism of action, a highly favourable risk/benefit ratio and low rate of adverse events [64], researchers will probably study the effects of NAC in muscle repair in the future.

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) have also shown beneficial effects in studies of muscle healing. ACE is a circulating enzyme that participates in the body's renin-angiotensin-aldosterone system (RAAS), which modulates extracellular volume and arterial vasoconstriction. Its inhibitors reduce morbidity, mortality, hospital admissions, and decline in physical function and exercise capacity in congestive heart failure patients. These therapeutic effects are attributed primarily to beneficial cardiovascular actions of these drugs [70]. Observations have linked pathologic fibrosis in various organ systems to the local effects of angiotensin II. The modulation of angiotensin II with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers has demonstrated decreased fibrosis and improved function in liver, kidney, and lung tissue [71-74]. Injured cardiac muscle also demonstrates dysfunction related to

fibrosis. Myocardium exposed to decreased levels of angiotensin II, either through the use of ACE inhibitors or ARBs, has also demonstrated a measurably improved function [75, 76]. However, it has been suggested that ACE inhibitor-induced positive effects may also be mediated by direct action on the skeletal muscle [70]. Recent studies reported on the beneficial side effects of ACE inhibitors in hypertensive patients free of chronic heart failure [70]. Treatment with ACE inhibitors was associated with better performance and muscular outcomes, and genetic studies also support the hypothesis that the ACE system may be involved in physical performance and skeletal muscle function [77, 78]. Moreover, elite athletes, particularly those in endurance sports, have also demonstrated findings consistent with inherent differences in their body's metabolism of angiotensin II, with decreased exposure resulting in improved skeletal muscle function [70, 79, 80]. In an *In vitro* and *In vivo* study by Bedair et al. angiotensin receptor blocker therapy significantly reduced fibrosis and led to an increase in the number of regenerating myofibres in acutely injured skeletal muscle and may therefore provide a safe, clinically available treatment for improving healing after skeletal muscle injury [81].

3. Future perspectives of cartilage tissue repair

Damage to articular cartilage is of great clinical consequence since the cartilage tissue comprises of limited intrinsic potential for healing due to the lack of blood supply and subsequent incomplete repair by local chondrocytes with inferior fibrocartilage formation. Surgical intervention is often the only option, but the repair of damaged cartilage is often less than satisfactory, and rarely restores full function or returns the tissue to its native normal state.

Tissue engineering of articular cartilage still remains challenging due to the special structure of cartilage tissue consisting of multiphasic cellular architecture and great weight-bearing characteristics. Good knowledge and understanding of cartilage structure, its metabolism, and the process of chondrogenesis enables *In vitro* cartilage production in terms of tissue engineering. The new concept of cartilage tissue preservation uses tissue-engineering technologies, combining new biomaterials as a scaffold, the application of growth factors, the use of stem cells, and mechanical stimulation. Scaffolds enable 3-dimensional environmental conditions to promote hyaline-like cartilage production. Additionally, various types of growth factors, which are the endogenous regulators of chondrogenesis, can be applied locally or in culture condition to promote cartilage development. Further studies are attempting to create the ideal scaffold and explore the synergistic effect of concomitant application of growth factors and mechanical loading. In clinical practice, new generations of autologous chondrocyte implantation (ACI) are based on the use of biodegradable materials that serve as temporary cell-carriers for the *In vitro* growth and subsequent implantation into the cartilage defect. Moreover, single stage procedures appear attractive, as they consist of natural chondral tissue inserted on the carrier and can reduce cost and patient morbidity since they avoid second operation and cell culturing procedures.

3.1. Tissue engineering of articular cartilage

The field of tissue engineering uses the principles of cell biology, engineering, and medicine in order to produce such a construct that can successfully replace damaged tissue. Engineered tissue should comprise of the characteristics of the native intact tissue in terms of histological structure, morphology, function, and mechanical properties. The challenges of tissue engineering of articular cartilage include isolating and culturing cells to gain relevant and reproducible constructs with good durability *In vivo*. A demanding and crucial role in the process of *In vitro* culturing represents phenotype regulation, *In vitro* expansion, scaffold design, the use of bioreactor, etc. All of these components should be optimized to advance cartilage tissue engineering from culturing to clinical application. In particular, there is still a need to develop suitable scaffolds that can provide a 3-dimensional environment for the cell to adhere to and adequately proliferate. Additionally, scaffolds should be mechanically strong and biocompatible. Cartilage tissue engineering usually uses bioactive molecules (growth factors) and mechanical loading to promote differentiation towards a cartilage phenotype [82].

3.1.1. Biomaterials and scaffolds

Scaffolds are engineered extracellular matrices that serve as an artificial structure capable of supporting 3-dimensional tissue formation. Cells are often implanted or seeded into these scaffolds and different biomaterials are used that allow cell attachment, growth, differentiation, and regeneration of functional cartilage tissue. Scaffolds were developed with the aim to improve the biological performance of chondrocytes as well as render the surgical technique easier. In cartilage tissue engineering, scaffolds should comprise of the following characteristics; they should be biocompatible (not triggering inflammatory response and not toxic), offering temporary support to cells, mechanically strong to protect cells and withstand *In vivo* forces during joint movement, and bioactive to provide cellular attachment and migration. Additionally, scaffolds should be biodegradable, serving as a temporary construct that is later replaced by a newly synthesized extracellular matrix (ECM) [83]. With time, the transplanted chondrocytes take over the function of the cell carrier; therefore they should be degraded once they have served their purpose. The ideal biodegradable scaffold should also enable uniform cell spreading possible [84-87].

In general, scaffolds are divided into natural material, synthetic polymers, and new materials. Natural materials include collagen, hyaluronic acid, fibrin glue, chitosan, agarose, and alginate. Their advantage is excellent biocompatibility since they are natural bodily constituents, thus degradation is physiological and non-toxic. On the other hand, their use includes sourcing, processing, and the risk of disease transmission. Synthetic polymers, especially PLA (poly alfa-hydroxil acid polymers) and PLGA (*poly lactic-co-glycolic acid*) are also widely used due to the approval of the FDA. Their major advantage is the design flexibility (highly porous 3-dimensional structure) and no risk of disease transmission. The disadvantages are acid degradation products, inflammatory response, and chronic inflammation due to high molecular weight proteins. Novel materials have been introduced recently, such as silk, cellulose, and other synthetic materials (biodegradable elastomer, polycaprolactone, poly

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(ether ester) copolymer scaffolds). Additionally, the combination of different materials is applied [88], e.g. gelatin and hyaluronic acid have been combined with a fibrin glue and chondroitin-6-suplphate. Furthermore, scaffolds to support bone formation (hydroxiapatite) were combined with the chitosan to enable the regeneration of osteochondral lesions.

The new approach represents the development of smart matrices that actively support cartilage formation and not only provide mechanical function but also allow control over cell metabolism, tissue formation, enable adjustment of the physical properties, inclusion of ECM motifs and active substances such as GF incorporated in microspheres to allow temporally and spatially controlled delivery of GF in scaffolds [87].

Although most of these developments seem to be promising for future clinical application, they are mainly used *In vitro* and in animal models [84-87]. Chondrocytes previously expanded and seeded onto scaffold produce a characteristic ECM rich in proteoglycans, collagen type II and aggrecan. After implantation in the full thickness femoral defect in rabbits, it promoted healing and regenerated a cartilage-like tissue [89]. The future prospective for cartilage repair is also based on the quality of integration between the newly formed tissue and the native tissue for achieving stable healing.

3.1.2. Growth factors

Growth factors and their signaling pathways are the essential regulators of chondrogenesis during tissue engineering and thus are the prime candidates for engineering of cartilage tissue. Chondrogenesis is a multistep process that comprises of several steps: precursor cell condensation, differentiation towards chondrogenic phenotype, secretion of cartilage specific ECM components (collagen type II, aggrecan and others), chondrocytes proliferation in the area of growth plate, further differentiation towards hypertrophy, and replacement of cartilage with the bone tissue. All of the steps are regulated by different and overlapping signals (Figure 1).

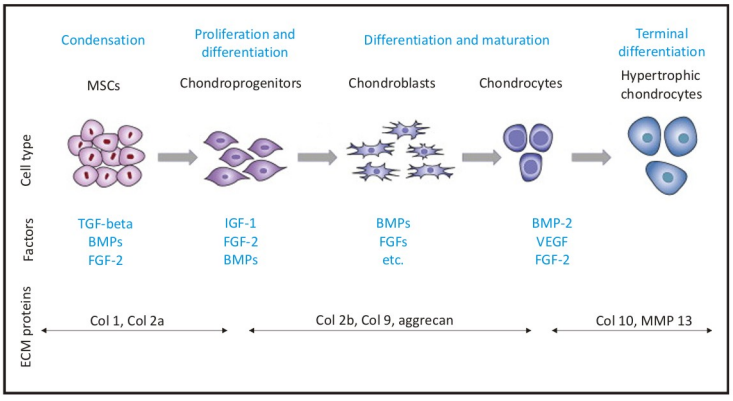

Figure 1. Schematic diagram of different stages of chondrogenesis (including main growth factors and alterations in ECM)

In general, GFs are endogenous regulators of chondrogenesis and their logical choice of use appears to be promising to stimulate anabolic responses and the repair of articular cartilage. For example, in *In vitro* cartilage formation it is essential to promote early chondrocytes differentiation and proliferation while trying to prevent further differentiation towards hypertrophy. However, the design and optimization of all GF's for a particular tissue engineering and/or local application is complex and has to consider the combination of different factors, their timing, concentrations, etc.

The most important factors currently used in tissue engineering are the members of transforming growth factor β (TGF-β) family, Bone Morphogenetic Protein (BMP), Insulin-like growth factors (IGF), Fibroblast Growth Factors (FGF), especially FGF-2 and FGF-18, Epidermal Growth Factor (EGF) and Vascular-Endothelial Growth Factors (VEGFs). The summary of the effect of different GFs on chondrocytes/cartilage is presented in Table 4. It is becoming increasingly apparent that GFs work synergistically and simultaneously to induce and promote cartilage formation; e.g.: TGF-β1 and FGF-2 [90] together with IGF-1 [91], BMP-7 and IGF-1 [91], TGFβ3 and BMP [92], etc. Based on the concept that several different GFs work in combination during cartilage repair, the use of PrP, autologous conditioned serum (ACS), and bone marrow concentrate were used in cartilage repair techniques [93].

	Growth factors	Effect on chondrocytes/cartilage
TGF-β	Transforming Growth Factor β	Stimulates synthesis of ECM Decreases catabolic activity
BMP-2	Bone Morphogenetic Protein - 2	Stimulates synthesis of ECM Increased ECM turnover (increased aggrecan degradation)
BMP-7	Bone Morphogenetic Protein - 7	Stimulates ECM synthesis Decrease cartilage degradation
IGF-1	Insulin-like growth factors	Stimulates ECM synthesis Decreases matrix catabolism
FGF-2	Fibroblast Growth Factors-2	Decreases aggrecanase activity Antagonizes PG synthesis
FGF-18	Fibroblast Growth Factors-18	Increases chondrocyte proliferation and stimulates ECM

Table 3. Proven effects of various growth factors on cartilage

3.1.3. Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are pluripotent cells found in multiple human adult tissues including bone marrow, synovial tissues, and adipose tissues. They are of great interest for scientists involved in cell therapy and tissue engineering since they have self-renewal capacity and multilineage differentiation potential. Depending on the cultivation conditions, they can differentiate into adipogenic, ostegenic or chondrogenic cells as well as form bone,

cartilage, and fat. Currently, researchers are exploring the possibilities of manipulating the stem cells under laboratory conditions into mature chondrocytes that can then be integrated into scaffolds for later application [94,95]. There are many studies reporting the isolation and characterization of MSCs from adult human synovium and periosteum and providing evidence about their multipotency at the single cell level [96, 97]. It was also demonstrated that human MSCs from different tissues possess distinctive biological properties [98]. Additionally, there is still the issue of whether MSCs are capable of forming stable hyaline-like cartilage as opposed to that formed during the process of endochondral ossification, which is later replaced with bone. The variability in biological responses of MSCs and no standardized MSC bioprocessing to obtain MSC preparations with consistent, reproducible, and quality-controlled biological potency for therapeutic applications limit the use of MSCs in clinical practice. On the other hand, the use of MSCs as chondrocyte substitutes in an ACI-equivalent procedure has become highly attractive since MCSs are easily accessible, easy to isolate and capable of expanding into culture as opposed to articular chondrocytes with limited proliferative capacity and rapid de-differentiation *In vitro*.

Furthermore, MCSs appear to be immune privileged under certain conditions [94-97]. Altogether, these properties would allow the generation of large batches of quality controlled MCSs preparations ready for allogenic use. In addition, limitations in patient-to-patient variability would be circumvented [93]. In animal models, MSCs have already shown significant potential for cartilage repair and novel approaches using MSCs as an alternative cell source to patient-derived chondrocytes are being tested [89, 99]. However, preclinical and clinical studies should be conducted in order to evaluate whether the implantation of MCSs results in a cartilage formation that is as durable as the one following the implantation of articular chondrocytes. Additionally, the application of MCSs can be further expanded to non-localized chronic lesions in osteoarthritis patients [100].

3.1.4. Mechanical load

A potential strategy in cartilage functional tissue engineering comprises of the effect of mechanical stimuli applied during *In vitro* tissue formation. Several studies demonstrated that mechanical forces stimulate the synthesis of ECM and may even enhance the mechanical properties of the developing tissue [101, 102]. After physical stimuli are applied to the tissue, the intracellular mechanisms convert mechanical signals into biochemical events responsible for regulating the transcription of genes governing cell growth and differentiation. This effect proved to be further intensified with concomitant application of growth factors and mechanical load in a synergistic manner [103, 104]. Various bioreactor systems have been developed in order to form cartilaginous grafts with similar biomechanical characteristics compared to native intact cartilage tissue [105-107]. Due to the complexity of the load and motion patterns within an articular joint, new bioreactors with multi-axial loading patterns are designed to recreate the *In vivo* situation [106, 107]. The interesting aspect of cartilage repair in clinical practice represents cell-based strategies that are not culture-intensive and allow single surgical procedure. Hence, a natural environment is the most suitable for tissue development therefore cell culture and bioreactors may not be required [108].

3.2. Clinical application of tissue engineering in cartilage repair

Cartilage repair has gained great interest since autologous cartilage implantation (ACI) has become an established treatment. The first line of treatment options remains microfracturing, due to low cost, arthroscopic procedure, and ease of performance.

Bone marrow stimulation techniques as well as ACI represent the cell-based approach for tissue regeneration in which the attendance of specific cells with the ability of proliferation and differentiation in desired cell phenotype plays a crucial role. In the case of bone marrow stimulating techniques, these cells are recruited from the bone marrow either by drilling or microfracturing; as such they are released from the medullar canal and subsequently form the blot clot on the side of the lesion. However, the final result is fibrous cartilage with inferior biomechanical properties compared to native hyaline cartilage. On the other hand, the histological analysis of random biopsy specimens after ACI procedure indicated the presence of type-II collagen and hyaline-like cartilage within the healing tissue.

ACI is both technically demanding and associated with a high percentage of reoperations. The modification of this cell therapy was designed to reduce complications such as periosteal hypertrophy, the need for second look arthroscopy, the development of fibrocartilage tissue with variable amount of hyaline cartilage, etc. The next generation of ACI was developed by replacing the periosteal patch with a biocompatible matrix and selecting cells of potentially improved chondrogenic potential. For example, second generation ACI uses collagen-covered autologous cultured chondrocyte implantation and in third-generation ACI, special cell carriers or cell-seeded scaffolds were created. Fourth generation cartilage repair focuses on growth factors and gene therapy, the use of stem cells and tissue engineering [109, 110]. In general, arthrotomy and a two-stage procedure are the most commonly used, but all-arthroscopic techniques and one-stage procedures (e.g. technique with minced articular cartilage) have become highly attractive treatment techniques. Additionally, preimplantation chondrocyte phenotype manipulation has also shown excellent outcomes.

3.2.1. Second generation ACI

Second generation ACI is still a two-step procedure, but in contrast to classical ACI it involves culturing in 3-dimensional conditions, which favours the maintenance of phenotypic stability of chondrocytes. In particular, chondrocytes are cultured on the scaffold that is biocompatible, enables cellular growth, and as such represents the graft to be transplanted. These scaffolds/matrices containing the chondrocytes are implanted on the chondral lesion and attached with fibrin glue. In this manner, periosteal grafts and their suturing onto healthy cartilage are not necessary. These techniques were developed in an attempt to resolve some of the most common problems indicated by the standard ACI technique such as periosteal hypertrophy, which is a source of complaints about localized pain among some patients.

The scaffolds used in second generation ACI should comprise all of the following futures: biocompatibility (no inflammatory response), biodegradability (controlled rate of degradation), bioactivity (promote maintenance of phenotype and proliferation), and permeability (to ensure nutrition). Natural and synthetic scaffolds can be used. The concern about the synthetic

scaffolds is the risk of the harmful effect of degradation products on surrounding tissue. A comparison of the first and second-generation ACI has shown rather equivalent short-term clinical outcomes, with similar complications and a similar rate of reoperation [110]. A variety of scaffolds have been introduced, implanted either through a small arthrotomy or arthroscopically and will be presented briefly: collagen-covered ACI (CACI or ACI-C), Hyalograft C based on hyaluronic acid and membrane/matrix induced ACI (MACI) and others.

The main innovation in CACI is the use of bioresorable collagen membrane cover instead of the periosteal cover. Initial reports showed clinical improvements of this second generation ACI with fewer complications compared to classical ACI. The clinical and functional assessment after two years showed that 74% of patients had good or excellent results following CACI compared to 67% after classical ACI (ACI-P or PACI – periosteal ACI). Revision arthroscopy was required in 36.4% in the PACI group one year after surgery due to shaving for hypertrophy compared to none in the CACI group [111]. In the systemic review of ACI procedures including 82 studies they reported that the failure rate was highest in PACI (7.7%) compared to CACI (1.5%). Similarly, the highest rate of unplanned re-operation was in the PACI group (27%) compared to CACI (5%) [112].

Matrix-induced ACI (MACI) was first introduced in 1998. In this technique, cells are seeded directly onto the surface of a biodegradable type I/III collagen membrane and as such overcome the shortcomings of the original periosteum-covered technique. The membrane is a bilayer structure, smooth on one side and rough and more porous on the inner side, with incorporated cells to stimulate cartilage matrix specific molecules. It was shown that chondrocytes can adhere and maintain their phenotypic characteristics while seeded onto a type I/III collagen membrane [113]. The procedure requires limited exposure of the joint ensuring shorter operation time and less morbidity. The rate of failure was low (0-6.3%) and mainly due to symptomatic graft hypertrophy or detachment. However, clinical, arthroscopic, and histological outcomes are comparable for CACI and MACI [114]. Additionally, MACI was also significantly more effective after two years compared to microfracturing [115]. Although significantly improved results after 5 years were reported, MACI still remains the cost-intensive alternative [116].

Hyalograft C implants autologous cells onto an esterified hyaluronic acid scaffold. It was reported that 76% of patients had no pain and 88% had no mobility problems. Additionally, 96% of patients' treated knee was assessed to be normal by the surgeon and cartilage repair was graded arthroscopically as normal or nearly normal in 96%. The majority of second-look biopsies showed hyaline-like cartilage and a very low rate of complications were recorded [117]. Several other studies also reported positive clinical results with Hyalograft C [118, 119]. Similarly, in two comparative studies, researchers found superiority over hyaluronic-acid based chondrocytes transplantation at five years follow up in respect to microfracture in young, active patients [120, 121].

3.2.2. Third and fourth generation ACI

Recently, further technological advances have led to a third-generation ACI, where chondrocytes are embedded into three-dimensionally constructed scaffolds (i.e. 3-dimensional

environment) for cell growth [122]. This novel approach uses: chondro-inductive or chondro-conductive matrix; autogenous or alogenous cells treated *In vitro* in order to induce cell proliferation, differentiation and production of ECM; a single-stage surgical approach; and mechanical stimulation to improve the material properties and maturation of the implant [123]. Such mature tissue might ensure shorter rehabilitation and shorten the time to achieve clinical efficiency. For example, the DeNovo Engineered Tissue (ET) graft (Zimmer, Warsaw) is generated from juvenile cartilage cells under special laboratory conditions and is hyaline-like. It is engineered by ISTO Technologies and the FDA approved ISTO's Investigational New Drug (IND) application for Neocartilage in 2006, which allowed them to pursue clinical trials of the product in humans.

Likewise, Neocart (Histogenics, Waltham, MA), a bioengineered tissue patch containing an autologous chondrocyte population matured in a biodegradable collagen matrix, uses bioreactor technology (hydrostatic pressure with modified flow rates and low oxygen) to stimulate ECM accumulation and suppress long-term degradation. A recent randomized study suggests that the safety of autologous cartilage tissue implantation, with the use of the NeoCart technique is similar to that of microfracture and associated with greater clinical efficacy at two years after treatment [124]. However, there are still technical problems remaining regarding the initial fixation technique, subchondral and edge integration, long-term durability, etc. [125].

A number of new generation ACI methods for implanting cultured autologous chondrocytes in a biodegradable matrix are currently in development or testing. These include Chondroselect (characterized chondrocyte implantation, TiGenex, Phase III trial), BioCart II (ProChon Biotech, Phase II trial), Cartilix (polymer hydrogel, Cartilix), MACI® (matrix-induced ACI, Verigen, available outside of the U.S.), Cartipatch (solid scaffold with an agarose-alginate matrix, TBF Tissue Engineering, Phase III trial), NeoCart (ACI with a 3-dimensional chondromatrix, Histogenics, Phase II trial) and Hyalograft C (ACI with a hyaluronic acid-based scaffold, Fidia Advanced Polymers). Although the clinical use of these second-generation ACI products has been reported in Europe, none are approved for use in the U.S. at this time [126].

The future of fourth generation cartilage repair focuses on gene therapy, the use of stem cells (bone marrow, adipose, or muscle derived) and tissue engineering. MSCs are an attractive cell source due to their differentiation capacities. To expand and deliver MSCs to the site of defect, the cells should be seeded into an appropriate scaffold that is biocompatible, mechanically stable, permeable, and biodegradable. A variety of biomaterials were introduced, e.g. carbohydrate polymers (hyaluronan, agarose, alginate, PLA/PLGA) that are protein-based (collagen, fibrin, gelatin) in order to obtain homogenous distribution within a 3-dimensional matrix.

Future generations of cartilage tissue engineering will also include methods to control the genome to direct chondrogenic differentiation towards a hyaline-like pathway. In this manner, the local cellular environment can be coordinated by a tightly regulated GFs that signal molecules to regulate cellular maturation and proliferation. Additionally, with the use of gene therapy, either viral or non-viral vectors can be applied into cells, which then express

chondrogenic GF. Gene transfer enables localized exposure of bioactive proteins or gene products to the site of tissue lesions. There have been numerous cDNAs cloned and used for biological stimulation of cartilage healing in terms of mitosis induction, synthesis of ECM components, induction of chondrogenesis by progenitor cells, inhibiting inflammatory response, etc. Researching involves identification and specific gene combinations that could be incorporated into vectors and delivered to target cells [127]. Current data indicates that efficient delivery and expression of certain genes may have an effect on overall healing response in cartilage tissue and is capable of turning the repair response towards the synthesis of a more hyaline cartilage tissue [128]. The novel approach in cartilage tissue engineering is the use of cell population certification (screening of gene markers, positive and negative factors, gene expression score - ChondroCelect), which enables prediction of whether cells are capable of making stable hyaline-like cartilage *In vitro*. By selecting those characterized cells with a high probability of maintaining a chondrogenic phenotype, the effectiveness of transplanted chondrocytes would be maximized.

3.2.3. One-step surgery

Recent directions in cartilage repair are moving towards the possibility of performing one-step surgery, including the use of MCS and GF, and to avoid the first surgery, harvesting cell material, and subsequent cell cultivation. Numerous studies reported that bone marrow stem cells are a useful source for restoring cartilage defects. Additionally, by the concomitant use of PRP and MCS it is possible to develop a single step procedure.

Single stage procedures can be divided into two categories: cell free implants (scaffolds) and cell-based implants (further subdivided according to the cell type utilized; auto- and allografts). One of the most common cell-free procedures is AMIC (autologous matrix-induced chondrogenesis). The technique requires a cell free implant that is “smart” enough to provide the appropriate stimuli to induce orderly and durable tissue regeneration. Moreover, it should be capable of inducing in situ cartilage formation. The AMIC procedure comprises of microfracturing combined with the implant of a porcine collagen type I/III bilayer matrix to stabilize blood clot formation.

The first reports on the AMIC technique were promising and the results were comparable to standard ACI with the advantage of a single stage technique and no donor site morbidity [129]. In a study with the mean follow up rate of 37 months, they reported highly satisfactory results in 87% with MRI showing moderate to complete filling and normal to hyperdense signal [130]. Another possibility is to use bone marrow concentrate (BMC) for MCS in treating cartilage defects. The technique consists of harvesting 40-60ml of bone marrow aspirate from the iliac crest, centrifugation, and the use of special enzymes to activate the BMC and produce the sticky clot material that is placed on the side of the lesion; finally the defect is covered with a collagen membrane.

An attractive option in terms of cell-based technologies is represented by minced articular cartilage procedures for repairing articular cartilage, as they are one-staged, autologous and inserted on scaffold carriers that provide chondro-milieu, mechanical protection and even distribution of the cells within the defect. The principle of the minced cartilage procedure is

to obtain hyaline-like "minced" cartilage pieces supplemented with the scaffold delivery system. Minced cartilage represents the source of cells and even relatively large defects can be treated with a small amount of cells; specifically, one-tenth of the cartilage that originally covered a defect is required. The proposed advantages of this procedure over conventional treatment are the elimination of the need for in-vitro cell expansion and a second surgical procedure. Several technologies are being investigated and are in current late stage trials [131]. The autograft cell-based procedure CAIC (Mitek, USA) is currently under phase III evaluation. During the procedure, autologous cartilage is harvested with the special shaver device, then morcellized and secured on resorbable polymer mesh with fibrin glue. DeNovo NT Graft ("Natural Tissue Graft", Zimmer, Warsaw) is a similar application used for treatment of the cartilage lesions limited to an articular surface with intact subchondral bone. It utilizes morcelized juvenile cartilage, which is secured with the fibrin glue. As there is no use of chemicals and minimal manipulation, a DeNovo NT Graft does not require FDA approval and is currently available in the United States. Both CAIS and DeNovo NT techniques rely on chondrocytes migration out of the cartilage tissue with subsequent matrix production to fill the defect [130-132]. Early animal and preclinical models have demonstrated hyaline-like cartilage. Clinical experience is limited, with short-term studies demonstrating both procedures to be safe, feasible, and effective, with improvements in subjective patient scores, and with magnetic resonance imaging [133].

4. Strategies to improve ligament and tendon repair

Tendons and ligaments are avascular and hypocellular with distinct mechanical features that make them difficult for currently available treatments to reach a complete functional repair of the damaged tissue. Tendon injuries, whether acute or chronic, are commonly managed either conservatively or surgically. Conservative management, such as rest, corticosteroid injection, orthotics, ultrasound, laser treatment, or shockwave provide pain relief but, when they fail, surgery is required [134].

Surgical repair may be indicated in acute injuries. In chronic lesions, excision of the involved area might be performed. However, repaired tendons have inferior properties when compared to healthy ones. The loss of mechanical features is mainly due to a distorted extra cellular matrix (ECM) composition and a misalignment of collagen fibrils of the scar tissue [134]. Another option is to use tendon or ligament grafts, but graft-augmentation devices and artificial prostheses have also been developed [135]. Because current treatment is suboptimal, alternative therapies have been developed, such as the delivery of growth factors, the development of engineered scaffolds or the application of stem cells.

4.1. Grafts and graft-augmentation devices

Autografts are used widely to repair the affected tendon and prevent instability due to the damaged ligament. The most commonly used autografts include hamstring tendons (semitendinosus and gracilis) and bone-patellar ligament (middle third)-bone. Several factors are

important in the selection of the graft tissue reconstruction, such as the initial mechanical properties of the graft tissue, morbidity resulting from graft harvesting, graft healing, and the initial mechanical properties of the graft fixation, [134].

Allografts represent an alternative option to autografts for tendon and ligament repair. Because of high cost, limited accessibility, associated risk of disease transmission and tissue rejection with the use of allografts, autografts are preferred.

Immediately after a reconstruction with autograft or allograft, the fixation site, not the graft midsubstance, is considered to be the weakest point; following that period, the process of ligamentization influences the mechanical properties of the graft, making it more vulnerable.

To prevent injuries of the graft until integration into the bone and the process of ligamentization is complete, graft augmentation devices were developed to provide immediate post-surgical protection. They share mechanical loads with the biological graft until the graft itself is capable of withstanding local tensile and compressive forces [134]. Graft augmentation devices should be resorbable, but the rate of resorption should be limited by gradual transfer of mechanical loads to the biological graft [134, 135].

4.2. Tissue engineering

Tissue engineering (TE) combines biological materials and cells into a construct that is eventually able to replace the regenerated tissue [136], through the merging of three areas: scaffold microenvironment, stem cells, and signalling biofactors. The goal is to reconstruct a ligament/tendon by providing a scaffold seeded with cell-inducing neotissue formation that adequately meets the required biological and mechanical properties [136,137]. Engineering fibrous tissues, such as tendons and ligaments, requires the use of fibre-based scaffolds, because they should possess appropriate mechanical properties to withstand high stresses, but also high porosity and surface area to allow the seeded cells to proliferate and regenerate the tissue [137].

4.2.1. Stem cells and scaffolds

The purpose of TE with responding cells is to induce a regenerative response instead of scarring. Tissue engineering can be divided into two subtypes: the *In vivo* approach and the *ex vivo-de novo*. The *In vivo* approach permits the self-regeneration of small tissue lesions [138]. The *ex vivo-de novo* approach is designed to produce functional tissue that can be implanted in the body. Several cells have been used: tenocytes, fibroblasts and stem cells. The latter can be derived from bone marrow, human tendons (ACL, PCL), adipose tissue or embryonic derived stem cells [138].

Upon injury, elongated fibroblast cells resident in the tendon are activated by the inflammatory response for collagen deposition. To conduct this function, tenocytes are assisted by tendon-derived stem cells (TDSCs) [139].

MSCs do not differentiate spontaneously during *In vitro* culture, which permits a controlled microenvironment, such as to dictate the differentiation of MSCs after implantation. Because they are more easily isolated and banked from bone marrow compared to TDSCs, they represent a more optimal source of stem cells suitable for therapeutic use. MSCs have been induced to differentiate to tenocytes through the Wnt signalling pathway and cyclic mechanical stimulation that mimics normal processes [140]. It was also found that platelet-rich plasma (PrP) stimulates both MSCs and TDSCs [139].

Adipose-derived stem cell (ASC) use for tendon regeneration and repair has recently been taken into consideration. In a recent study, the role of these stem cells in primary tendon healing has been investigated by a local autologous ASC-mixed platelet-rich plasma (PrP) application at the site of tendon injury in a control to PrP application only [141]. The tensile strengths experimental groups were found to be significantly higher in comparison to the control group and, along with higher expression of collagen type I, FGF and VEGF levels in the experimental group, ASCs seems to enhance primary tendon healing.

It is now well accepted that seeded grafts vastly improve outcomes over un-seeded grafts. Recently, collagen matrices cultured with MSCs have appeared on the horizon for tendon repair [142-144]. The isoelectric focusing technique aligns collagen fibres to the parameters of the target tissue, adjusting the density, alignment, and strength of dense connective tissue (Gurkan: Comparison of morphology, orientation, and migration of tendon derived fibroblasts and bone marrow stromal cells on electrochemically aligned collagen constructs 2010). These matrices support a higher proliferation rate of MSCs compared to randomly oriented collagen. Currently, the versatility of synthetic polymers shows great promise in tissue engineering. Poly (1.8 octanediol-co-citrate) scaffold (POC) is a highly reproducible elastomeric material capable of being used as a synthetic scaffold to support cell growth. Instead of attaching tendinous grafts to bone via screws, the optimal approach is reconstruction using the collaboration of synthetic materials with MSCs. Paradoxically, the very complexity of the fibrocartilage interface makes it a perfect candidate for POC utilization. A scaffold with three distinct regions would allow formation of a collagenous tendon along one edge, osseous material along the other, and a middle zone representing the transition from tendon to bone. Given the capacity of MSCs to differentiate into osteogenic and tenogenic lineages, a single cell population seeded onto the scaffold could regenerate the complex fibrocartilage interface. Additionally, POC scaffolds could be crafted according to the target tendon interface, relying on Wolff's Law to govern the dynamics and load of the tendon aimed for reconstruction [139].

4.2.2. Bioreactors

A bioreactor in TE is a device that simulates a physiological environment in order to promote cell or tissue growth In vivo. Tendons respond to mechanical forces by changing the metabolism as well as their structural and mechanical properties. Without the appropriate biomechanical stimulation, newly formed tissue will lack appropriate collagenous organization and alignment for sufficient load-bearing capacity [134, 145, 146]. When subjected to

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mechanical stimulation In vitro, embryonic stem cells exhibited tenocyte-like morphology and positively expressed tendon-related gene markers, as well as other mechanosensory structures and molecules (cilia, integrins and myosin). In ectopic transplantation, the TE tendon under In vivo mechanical stimulus displayed more regularly aligned cells and larger collagen fibres that enabled enhanced tendon regeneration in situ, as evidenced by better histological scores and superior mechanical performance characteristics [145]. In a recent study, rabbit flexor tendons were deprived of cells and exposed to cyclic strain in a bioreactor, in comparison to a control, which was kept unloaded in a medium for 5 days [147]. The tendons were then implanted to bridge a zone II defect in the rabbit, followed by determination of ultimate tensile strength and elastic modulus after 4 weeks. Both were significantly improved in tendon constructs that were exposed to cyclic strain, and the histology showed an increased cellularity in the bioreactor tendons. In another study, it was showed that the material properties of human allograft tissue-engineered constructs can be enhanced by reseeding and dynamic conditioning [148]. It was found that while conditioning duration has a significant effect on material properties, the load magnitude does not. The issue of attrition in biomechanical properties with time following cycle completion must be addressed before bioreactor preconditioning can be successfully introduced as a step in the processing of these constructs for clinical application.

4.2.3. Growth factors

Following acute tendon injury, circulation-derived cells play a crucial role in the healing processes of tissue. It was shown, that locally injected PrP is useful as an activator of circulation-derived cells for the enhancement of the initial tendon healing process [149]. PrP also improves the mechanical properties of tendons in the early phase following acute injury, in terms of increase in the force at failure, ultimate stress, and stiffness; but the effect seems to vanish in the long-term follow up [150]. To date, there is still a debate regarding the positive effect of PrP following acute tendon injury. There are studies that confirm the positive effect of PrP on tendon healing, since an earlier return to sports, decreased cross-sectional area of tendon, and improved earlier range of ankle motion, following Achilles tendon reconstruction was noted [151]. It is speculated that *In vivo* use of PrP, as well as platelet-poor plasma to a certain extent, in tendon injuries might accelerate the catabolic demarcation of traumatically injured tendon matrices and promote angiogenesis and formation of a fibrovascular callus [152]. A study showed that platelets influence only the early phases of regeneration, but this allows for mechanical stimulation to start driving neo-tendon development at an earlier time point, which kept it constantly ahead of the controls [153]. However, all studies do not confirm the positive effect of PrP; in fact a possible negative effect of PrP on the functional results after the reconstruction of an Achilles tendon during a long-term follow up was observed [154].

In chronic tendon lesions, especially tendinopathy, the use of PrP is focused on restoring normal tissue composition while avoiding further degeneration. Ultrasound-guided injections of PrP were effective in reducing pain in elbow tendinosis, medial epicondylitis [155] and jumper's knee [156]. Until now, few high-quality studies on the use of autologous GF

injections for the management of chronic tendinopathy showed no significant improvement compared to a control group, but in those studies, autologous blood was injected and not PrP [157-159]. Currently, there is level 3 (limited) evidence that PrP injections improve pain or function in chronic tendinopathy [160]. More research on basic science and the clinical application of PrP needs to be undertaken before a final recommendation for PrP administration for the treatment of tendinosis can be made [47, 160].

A study performed at our department showed that the administration of PrP when reconstructing ACL with a hamstring autograft enhances early graft revascularization in the interface zone between graft and bone in the tibial tunnel; furthermore, PrP stimulates the formation of a sclerotic bony ring around the graft [161]. Platelet-leukocyte gel, applied locally, can also improve knee stability in the first three-month period and especially in the second three-month period [162]. Studies indicate that the delivery of PrP mimics and accelerates physiological healing and reparative tissue processes in graft healing and graft ligamentization process. Therefore, such therapy could improve knee stability and shorten the period of rehabilitation after reconstructive knee surgery. However, not all studies on humans confirm the positive effect of PrP. In one study after ACL reconstruction using patellar tendon graft with the application of PrP, researchers did not find any statistically important difference in inflammatory parameters, appearance of the graft on MRI, or clinical evaluation using validated scores [163]. Still others did not find any differences in graft fixation after ACL reconstruction with hamstring allograft and application of PrP [164].

4.3. Extracorporeal shock wave therapy

Extracorporeal shock wave therapy (ESWT) is a technique used in the treatment of tendon disorders, particularly calcific tendinopathy. The treatment is an extension of renal lithotripsy. It is a non-invasive modality used to stimulate healing, particularly in ligament, tendon, or bone structures. A high-energy sound wave rapidly increases pressure as it travels through the tissue, which results in cavitation that causes microtrauma. This stimulates an increase in blood flow and new blood vessel formation in the target area. Studies showed an increase in inflammatory cytokines and growth factors, as well as the regulation of tumour necrosis factor, interleukin, and bone morphogenetic protein following ESWT. Studies indicate that differentiated tenocytes are metabolically ''activated'' by ESWT and significantly induced proliferation and production of collagen (mainly type I) compared with untreated cells [165, 166]. Not all studies were able to show a positive effect of ESWT, but this was later argued to be a possible consequence of topical anaesthetics that interfere with ESWT treatment [167].

Numerous other substances have been used in the treatment of tendon disorders, including sclerosants, calcium gluconate, heparin, dextrose, and aprotinin; however, more studies have to be performed to prove their efficiency [168]. To date, no optimal treatment modalities for injured tendons or ligaments have been proposed. In fact, sheathed tendons may heal differently from those not enclosed in sheaths and the process of healing of an intra-articular ligament may differ from an extra-articular ligament. Recent studies support the idea that scaffolds can provide an alternative for tendon augmentation and that tissue engi-

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neering has an enormous therapeutic potential. In recent years studies revealed that tendon healing and regeneration may be improved by the application of several growth factors and the use of PrP expanded widely. Today, many different producers provide PrP of different composition that makes studies hard to compare. Future studies will have to explain which concentrations of PrP works the best, where it is effective and what the role of accompanying leucocytes is.

5. Conclusion

Regenerative medicine holds great promise for sports medicine with aim to develop novel therapies that will replace, repair, or promote tissue regeneration. It is an increasingly expanding area of research with hopes of providing therapeutic treatments for diseases and/or injuries that conventional medicines cannot effectively treat. Skeletal muscle has a great self-regenerative capacity, but it is unfortunately limited by fibrotic infiltration. Although none of the antifibrotic agents to improve skeletal muscle regeneration have been tested on humans to date, its clinical implications are potentially far-reaching and include not only sports-related injuries, but also diseases such as muscular dystrophies and trauma- and surgery-related injury. With emerging novel therapeutic targets this is an important area of research and presents a basis for further possibilities to study different mechanisms of action and effects drug combinations for improving muscle regeneration.

Biomaterials play an important role in directing tissue growth and may provide another tool to manipulate and control stem cell behaviour. Growth factors and therapies using mesenchymal stem cells, scaffolds, and tissue engineering using bioreactors represent promising strategies for tendon, ligament and cartilage repair. While therapies using growth factors seem to be well established in case of the first two, lack of scientific evidence still makes them questionable. In the future of cartilage repair, the modification of cellular differentiation following microfracture could be alternated with the use of exogenous growth factors and scaffolds in order to retain chongrogenic phenotype and to improve the quality of repair tissue generated in the defect. The important future prospective of cartilage repair is also focused on the quality of the bonding and integration of the newly engineered tissue to native cartilage to achieve stable healing. This holds potential for tissue-engineered strategies that would enable repairing complex cartilage lesions together with the subchondral bone and other structures. However, as with all innovations, carefully conducted studies should be carried out to access the efficiency for cartilage regeneration. Furthermore, long term prospective randomized studies are needed to confirm the encouraging preliminary results.

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References

[1] Gehrig SM, Lynch GS. Emerging drugs for treating skeletal muscle injury and promoting muscle repair. Expert Opin Emerg Dr. 2011 Mar;16(1):163-82.

[2] Bates SJ, Morrow E, Zhang AY, Pham H, Longaker MT, Chang J. Mannose-6-phosphate, an inhibitor of transforming growth factor-beta, improves range of motion after flexor tendon repair. J Bone Joint Surg Am. 2006 Nov;88A(11):2465-72.

[3] Mishra DK, Friden J, Schmitz MC, Lieber RL. Anti-inflammatory medication after muscle injury. A treatment resulting in short-term improvement but subsequent loss of muscle function. J Bone Joint Surg Am. 1995 Oct;77(10):1510-9.

[4] Shen W, Li Y, Tang Y, Cummins J, Huard J. NS-398, a cyclooxygenase-2-specific inhibitor, delays skeletal muscle healing by decreasing regeneration and promoting fibrosis. Am J Pathol. 2005 Oct;167(4):1105-17.

[5] Nakatani M, Takehara Y, Sugino H, Matsumoto M, Hashimoto O, Hasegawa Y, et al. Transgenic expression of a myostatin inhibitor derived from follistatin increases skeletal muscle mass and ameliorates dystrophic pathology in mdx mice. Faseb Journal. 2008 Feb;22(2):477-87.

[6] Amthor H, Connolly D, Patel K, Brand-Saberi B, Wilkinson DG, Cooke J, et al. The expression and regulation of follistatin and a follistatin-like gene during avian somite compartmentalization and myogenesis. Dev Biol. 1996 Sep 15;178(2):343-62.

[7] McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature. 1997 May 1;387(6628):83-90.

[8] Findlay JK. An update on the roles of inhibin, activin, and follistatin as local regulators of folliculogenesis. Biol Reprod. 1993 Jan;48(1):15-23.

[9] Zhu J, Li Y, Lu A, Gharaibeh B, Ma J, Kobayashi T, et al. Follistatin Improves Skeletal Muscle Healing after Injury and Disease through an Interaction with Muscle Regeneration, Angiogenesis, and Fibrosis. Am J Pathol. 2011 May 31.

[10] Amthor H, Nicholas G, McKinnell I, Kemp CF, Sharma M, Kambadur R, et al. Follistatin complexes Myostatin and antagonises Myostatin-mediated inhibition of myogenesis. Dev Biol. 2004 Jun 1;270(1):19-30.

[11] Foley JW, Bercury SD, Finn P, Cheng SH, Scheule RK, Ziegler RJ. Evaluation of systemic follistatin as an adjuvant to stimulate muscle repair and improve motor function in Pompe mice. Mol Ther. 2010 Sep;18(9):1584-91.

[12] Gilson H, Schakman O, Kalista S, Lause P, Tsuchida K, Thissen JP. Follistatin induces muscle hypertrophy through satellite cell proliferation and inhibition of both myostatin and activin. Am J Physiol Endocrinol Metab. 2009 Jul;297(1):E157-64.

[13] Lee SJ, McPherron AC. Regulation of myostatin activity and muscle growth. P Natl Acad Sci USA. 2001 Jul 31;98(16):9306-11.

[14] Kota J, Handy CR, Haidet AM, Montgomery CL, Eagle A, Rodino-Klapac LR, et al. Follistatin gene delivery enhances muscle growth and strength in nonhuman primates. Sci Transl Med. 2009 Nov 11;1(6):6ra15.

[15] Hiroki E, Abe S, Iwanuma O, Sakiyama K, Yanagisawa N, Shiozaki K, et al. A comparative study of myostatin, follistatin and decorin expression in muscle of different origin. Anat Sci Int. 2011 Mar 18.

[16] Hocking AM, Shinomura T, McQuillan DJ. Leucine-rich repeat glycoproteins of the extracellular matrix. Matrix Biol. 1998 Apr;17(1):1-19.

[17] Nishimura T, Futami E, Taneichi A, Mori T, Hattori A. Decorin expression during development of bovine skeletal muscle and its role in morphogenesis of the intramuscular connective tissue. Cells Tissues Organs. 2002;171(2-3):199-214.

[18] Stander M, Naumann U, Dumitrescu L, Heneka M, Loschmann P, Gulbins E, et al. Decorin gene transfer-mediated suppression of TGF-beta synthesis abrogates experimental malignant glioma growth in vivo. Gene Ther. 1998 Sep;5(9):1187-94.

[19] Ungefroren H, Ergun S, Krull NB, Holstein AF. Expression of the Small Proteoglycans Biglycan and Decorin in the Adult Human Testis. Biol Reprod. 1995 May;52(5): 1095-105.

[20] Fukushima K, Badlani N, Usas A, Riano F, Fu FH, Huard J. The use of an antifibrosis agent to improve muscle recovery after laceration. Am J Sport Med. 2001 Jul-Aug; 29(4):394-402.

[21] Li Y, Foster W, Deasy BM, Chan Y, Prisk V, Tang Y, et al. Transforming growth factor-beta1 induces the differentiation of myogenic cells into fibrotic cells in injured skeletal muscle: a key event in muscle fibrogenesis. Am J Pathol. 2004 Mar;164(3): 1007-19.

[22] Sato K, Li Y, Foster W, Fukushima K, Badlani N, Adachi N, et al. Improvement of muscle healing through enhancement of muscle regeneration and prevention of fibrosis. Muscle Nerve. 2003 Sep;28(3):365-72.

[23] Li Y, Li J, Zhu J, Sun B, Branca M, Tang Y, et al. Decorin gene transfer promotes muscle cell differentiation and muscle regeneration. Mol Ther. 2007 Sep;15(9):1616-22.

[24] Kishioka Y, Thomas M, Wakamatsu JI, Hattori A, Sharma M, Kambadur R, et al. Decorin enhances the proliferation and differentiation of myogenic cells through suppressing myostatin activity. J Cell Physiol. 2008 Jun;215(3):856-67.

[25] Miura T, Kishioka Y, Wakamatsu J, Hattori A, Hennebry A, Berry CJ, et al. Decorin binds myostatin and modulates its activity to muscle cells. Biochem Biophys Res Commun. 2006 Feb 10;340(2):675-80.

[26] Zhu J, Li Y, Shen W, Qiao C, Ambrosio F, Lavasani M, et al. Relationships between transforming growth factor-beta 1, myostatin, and decorin - Implications for skeletal muscle fibrosis. J Biol Chem. 2007 Aug 31;282(35):25852-63.

[27] Wheelock EF. Interferon-Like Virus-Inhibitor Induced in Human Leukocytes by Phytohemagglutinin. Science. 1965 Jul 16;149(3681):310-1.

[28] Farrar MA, Schreiber RD. The molecular cell biology of interferon-gamma and its receptor. Annu Rev Immunol. 1993;11:571-611.

[29] Cheng M, Nguyen MH, Fantuzzi G, Koh TJ. Endogenous interferon-gamma is required for efficient skeletal muscle regeneration. Am J Physiol Cell Physiol. 2008 May;294(5):C1183-91.

[30] Foster W, Li Y, Usas A, Somogyi G, Huard J. Gamma interferon as an antifibrosis agent in skeletal muscle. J Orthop Res. 2003 Sep;21(5):798-804.

[31] Ulloa L, Doody J, Massague J. Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/STAT pathway. Nature. 1999 Feb 25;397(6721): 710-3.

[32] Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. FASEB J. 2004 May;18(7):816-27.

[33] Shelton GD, Calcutt NA, Garrett RS, Gu D, Sarvetnick N, Campana WM, et al. Necrotizing myopathy induced by overexpression of interferon-gamma in transgenic mice. Muscle Nerve. 1999 Feb;22(2):156-65.

[34] Chen JW, Chen SY, Li HY, Shang XL, Wu ZY. [Effect of exogenous interferon gamma on the healing of injured skeletal muscle following injury]. Zhongguo Gu Shang. 2008 Jun;21(6):434-7.

[35] Schrell UM, Gauer S, Kiesewetter F, Bickel A, Hren J, Adams EF, et al. Inhibition of proliferation of human cerebral meningioma cells by suramin: effects on cell growth, cell cycle phases, extracellular growth factors, and PDGF-BB autocrine growth loop. J Neurosurg. 1995 Apr;82(4):600-7.

[36] Zumkeller W, Schofield PN. Growth factors, cytokines and soluble forms of receptor molecules in cancer patients. Anticancer Res. 1995 Mar-Apr;15(2):343-8.

[37] Liu N, Tolbert E, Ponnusamy M, Yan H, Zhuang S. Delayed administration of suramin attenuates the progression of renal fibrosis in obstructive nephropathy. J Pharmacol Exp Ther. 2011 May 27.

[38] Mietz H, Krieglstein GK. Suramin to enhance glaucoma filtering procedures: a clinical comparison with mitomycin. Ophthalmic Surg Lasers. 2001 Sep-Oct;32(5):358-69.

[39] Chan YS, Li Y, Foster W, Fu FH, Huard J. The use of suramin, an antifibrotic agent, to improve muscle recovery after strain injury. Am J Sport Med. 2005 Jan;33(1):43-51.

[40] Taniguti AP, Pertille A, Matsumura CY, Santo Neto H, Marques MJ. Prevention of muscle fibrosis and myonecrosis in mdx mice by suramin, a TGF-beta1 blocker. Muscle Nerve. 2011 Jan;43(1):82-7.

[41] Masterson R, Hewitson TD, Kelynack K, Martic M, Parry L, Bathgate R, et al. Relaxin down-regulates renal fibroblast function and promotes matrix remodelling in vitro. Nephrol Dial Transpl. 2004 Mar;19(3):544-52.

[42] Samuel CS, Unemori EN, Mookerjee I, Bathgate RAD, Layfield SL, Mak J, et al. Relaxin modulates cardiac fibroblast proliferation, differentiation and collagen production and reverses cardiac fibrosis in vivo. Endocrinology. 2004 Sep;145(9):4125-33.

[43] Unemori EN, Pickford LB, Salles AL, Piercy CE, Grove BH, Erikson ME, et al. Relaxin induces an extracellular matrix-degrading phenotype in human lung fibroblasts in vitro and inhibits lung fibrosis in a murine model in vivo. J Clin Invest. 1996 Dec 15;98(12):2739-45.

[44] Bathgate RA, Lekgabe ED, McGuane JT, Su Y, Pham T, Ferraro T, et al. Adenovirus-mediated delivery of relaxin reverses cardiac fibrosis. Mol Cell Endocrinol. 2008 Jan 2;280(1-2):30-8.

[45] Mu X, Urso ML, Murray K, Fu F, Li Y. Relaxin regulates MMP expression and promotes satellite cell mobilization during muscle healing in both young and aged mice. Am J Pathol. 2010 Nov;177(5):2399-410.

[46] Official WADA Website. 2012; Available from: http://www.wada-ama.org/en/Media-Center/Archives/Articles/WADA-2011-Prohibited-List-Now-Published/.

[47] International Olympic Commitee: IOC Consensus Statement on the use of platelet-rich plasma (PRP) in sports medicine. http://www.olympic.org/Documents/Reports/EN/IOC_PRP_Consensus_Statement-ENG.pdf.

[48] Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: from basic science to clinical applications. Am J Sports Med. 2009 Nov;37(11):2259-72.

[49] Sanchez M, Anitua E, Orive G, Mujika I, Andia I. Platelet-rich therapies in the treatment of orthopaedic sport injuries. Sports medicine. 2009;39(5):345-54.

[50] Bachl N, Derman W, Engebretsen L, Goldspink G, Kinzlbauer M, Tschan H, et al. Therapeutic use of growth factors in the musculoskeletal system in sports-related injuries. The Journal of sports medicine and physical fitness. 2009 Dec;49(4):346-57.

[51] Hildebrand KA, Woo SL, Smith DW, Allen CR, Deie M, Taylor BJ, et al. The effects of platelet-derived growth factor-BB on healing of the rabbit medial collateral ligament. An in vivo study. The American journal of sports medicine. 1998 Jul-Aug;26(4):549-54.

[52] Batten ML, Hansen JC, Dahners LE. Influence of dosage and timing of application of platelet-derived growth factor on early healing of the rat medial collateral ligament. Journal of orthopaedic research: official publication of the Orthopaedic Research Society. 1996 Sep;14(5):736-41.

[53] Murray MM, Spindler KP, Abreu E, Muller JA, Nedder A, Kelly M, et al. Collagen-platelet rich plasma hydrogel enhances primary repair of the porcine anterior cruci-

ate ligament. Journal of orthopaedic research: official publication of the Orthopaedic Research Society. 2007 Jan;25(1):81-91.

[54] Wang-Saegusa A, Cugat R, Ares O, Seijas R, Cusco X, Garcia-Balletbo M. Infiltration of plasma rich in growth factors for osteoarthritis of the knee; short-term effects on function and quality of life. Archives of orthopaedic and trauma surgery. 2011 Mar; 131(3):311-7.

[55] Shen W, Li Y, Zhu JH, Schwendener R, Huard J. Interaction between macrophages, TGF-beta 1, and the COX-2 pathway during the inflammatory phase of skeletal muscle healing after injury. J Cell Physiol. 2008 Feb;214(2):405-12.

[56] Menetrey J, Kasemkijwattana C, Day CS, Bosch P, Vogt M, Fu FH, et al. Growth factors improve muscle healing in vivo. J Bone Joint Surg Br. 2000 Jan;82B(1):131-7.

[57] Wright-Carpenter T, Klein P, Schaferhoff P, Appell HJ, Mir LM, Wehling P. Treatment of muscle injuries by local administration of autologous conditioned serum: A pilot study on sportsmen with muscle strains. Int J Sports Med. 2004 Nov;25(8): 588-93.

[58] Kelc R, Trapecar, M., Gradisnik, L., Mlakar, R., Rupnik, MS., Cencic, A., Vogrin, M. New therapeutic strategy for muscle repair after injury: platelet-rich plasma and TGF-ß antagonists. Poster presentation. Development, function and repair of the muscle cell: Frontiers in myogenesis; New York, USA2011.

[59] Mishra A, Woodall J, Vieira A. Treatment of Tendon and Muscle Using Platelet-Rich Plasma. Clin Sport Med. 2009 Jan;28(1):113-+.

[60] Dennis PA, Rifkin DB. Cellular activation of latent transforming growth factor beta requires binding to the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor. Proc Natl Acad Sci U S A. 1991 Jan 15;88(2):580-4.

[61] Roberts A, Sporn, MB. Transforming growth factor-beta. Clark R, editor. New York: Plenum; 1996.

[62] Angunawela RI, Marshall J. Inhibition of transforming growth factor-beta1 and its effects on human corneal fibroblasts by mannose-6-phosphate. Potential for preventing haze after refractive surgery. J Cataract Refract Surg. 2010 Jan;36(1):121-6.

[63] Yang R, Xia C, Wang X, Sun K, Yang X, Tian S, et al. [Effects of mannose-6-phosphate on transforming growth factor beta and transforming growth factor beta receptor expression of flexor tendon cells]. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi. 2010 Jan;24(1):64-8.

[64] Millea PJ. N-acetylcysteine: multiple clinical applications. Am Fam Physician. 2009 Aug 1;80(3):265-9.

[65] Liu RM, Liu Y, Forman HJ, Olman M, Tarpey MM. Glutathione regulates transforming growth factor-beta-stimulated collagen production in fibroblasts. Am J Physiol Lung Cell Mol Physiol. 2004 Jan;286(1):L121-8.

[66] Hagiwara SI, Ishii Y, Kitamura S. Aerosolized administration of N-acetylcysteine attenuates lung fibrosis induced by bleomycin in mice. Am J Respir Crit Care Med. 2000 Jul;162(1):225-31.

[67] Meurer SK, Lahme B, Tihaa L, Weiskirchen R, Gressner AM. N-acetyl-L-cysteine suppresses TGF-beta signaling at distinct molecular steps: the biochemical and biological efficacy of a multifunctional, antifibrotic drug. Biochem Pharmacol. 2005 Oct 1;70(7): 1026-34.

[68] Sugiura H, Ichikawa T, Liu X, Kobayashi T, Wang XQ, Kawasaki S, et al. N-acetyl-L-cysteine inhibits TGF-beta1-induced profibrotic responses in fibroblasts. Pulm Pharmacol Ther. 2009 Dec;22(6):487-91.

[69] Marian AJ, Senthil V, Chen SN, Lombardi R. Antifibrotic effects of antioxidant N-acetylcysteine in a mouse model of human hypertrophic cardiomyopathy mutation. J Am Coll Cardiol. 2006 Feb 21;47(4):827-34.

[70] Onder G, Vedova CD, Pahor M. Effects of ACE inhibitors on skeletal muscle. Curr Pharm Des. 2006;12(16):2057-64.

[71] Lim DS, Lutucuta S, Bachireddy P, Youker K, Evans A, Entman M, et al. Angiotensin II blockade reverses myocardial fibrosis in a transgenic mouse model of human hypertrophic cardiomyopathy. Circulation. 2001 Feb 13;103(6):789-91.

[72] Otsuka M, Takahashi H, Shiratori M, Chiba H, Abe S. Reduction of bleomycin induced lung fibrosis by candesartan cilexetil, an angiotensin II type 1 receptor antagonist. Thorax. 2004 Jan;59(1):31-8.

[73] Paizis G, Gilbert RE, Cooper ME, Murthi P, Schembri JM, Wu LL, et al. Effect of angiotensin II type 1 receptor blockade on experimental hepatic fibrogenesis. J Hepatol. 2001 Sep;35(3):376-85.

[74] Suga S, Mazzali M, Ray PE, Kang DH, Johnson RJ. Angiotensin II type 1 receptor blockade ameliorates tubulointerstitial injury induced by chronic potassium deficiency. Kidney Int. 2002 Mar;61(3):951-8.

[75] Gremmler B, Kunert M, Schleiting H, Ulbricht LJ. Improvement of cardiac output in patients with severe heart failure by use of ACE-inhibitors combined with the AT1-antagonist eprosartan. Eur J Heart Fail. 2000 Jun;2(2):183-7.

[76] Swedberg K, Kjekshus J. Effects of enalapril on mortality in severe congestive heart failure: results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). Am J Cardiol. 1988 Jul 11;62(2):60A-6A.

[77] Savo A, Maiorano PM, Onder G, Bernabei R. Pharmacoepidemiology and disability in older adults: can medications slow the age-related decline in physical function? Expert Opin Pharmacother. 2004 Feb;5(2):407-13.

[78] Jones A, Woods DR. Skeletal muscle RAS and exercise performance. Int J Biochem Cell Biol. 2003 Jun;35(6):855-66.

[79] Frederiksen H, Bathum L, Worm C, Christensen K, Puggaard L. ACE genotype and physical training effects: a randomized study among elderly Danes. Aging Clin Exp Res. 2003 Aug;15(4):284-91.

[80] Frederiksen H, Gaist D, Bathum L, Andersen K, McGue M, Vaupel JW, et al. Angiotensin I-converting enzyme (ACE) gene polymorphism in relation to physical performance, cognition and survival--a follow-up study of elderly Danish twins. Ann Epidemiol. 2003 Jan;13(1):57-65.

[81] Bedair HS, Karthikeyan T, Quintero A, Li Y, Huard J. Angiotensin II receptor blockade administered after injury improves muscle regeneration and decreases fibrosis in normal skeletal muscle. Am J Sports Med. 2008 Aug;36(8):1548-54.

[82] Hildner F, Albrecht C, Gabriel C, Redl H, van Griensven M. State of the art and future perspectives of articular cartilage regeneration: a focus on adipose-derived stem cells and platelet-derived products. J Tissue Eng Regen Med. 2011 Apr;5(4):e36-51.

[83] Tuli R, Li WJ, Tuan RS. Current state of cartilage tissue engineering. Arthritis research & therapy. [Review]. 2003;5(5):235-8.

[84] Taboas JM, Maddox RD, Krebsbach PH, Hollister SJ. Indirect solid free form fabrication of local and global porous, biomimetic and composite 3D polymer-ceramic scaffolds. Biomaterials. 2003 Jan;24(1):181-94.

[85] Stoop R. Smart biomaterials for tissue engineering of cartilage. Injury. 2008 Apr;39 Suppl 1:S77-87.

[86] Moutos FT, Guilak F. Composite scaffolds for cartilage tissue engineering. Biorheology. 2008;45(3-4):501-12.

[87] Wang X, Wenk E, Zhang X, Meinel L, Vunjak-Novakovic G, Kaplan DL. Growth factor gradients via microsphere delivery in biopolymer scaffolds for osteochondral tissue engineering. J Control Release. 2009 Mar 4;134(2):81-90.

[88] Ameer GA, Mahmood TA, Langer R. A biodegradable composite scaffold for cell transplantation. J Orthop Res. 2002 Jan;20(1):16-9.

[89] Grigolo B, Lisignoli G, Desando G, Cavallo C, Marconi E, Tschon M, et al. Osteoarthritis treated with mesenchymal stem cells on hyaluronan-based scaffold in rabbit. Tissue engineering Part C, Methods. [Research Support, Non-U.S. Gov't]. 2009 Dec; 15(4):647-58.

[90] Pei M, He F, Vunjak-Novakovic G. Synovium-derived stem cell-based chondrogenesis. Differentiation. 2008 Dec;76(10):1044-56.

[91] Cals FL, Hellingman CA, Koevoet W, Baatenburg de Jong RJ, van Osch GJ. Effects of transforming growth factor-beta subtypes on in vitro cartilage production and mineralization of human bone marrow stromal-derived mesenchymal stem cells. J Tissue Eng Regen Med. 2012 Jan;6(1):68-76.

[92] Rui YF, Du L, Wang Y, Lui PP, Tang TT, Chan KM, et al. Bone morphogenetic protein 2 promotes transforming growth factor beta3-induced chondrogenesis of human osteoarthritic synovium-derived stem cells. Chin Med J (Engl). 2010 Nov;123(21): 3040-8.

[93] Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. Clin Orthop Relat Res. [Review]. 2011 Oct;469(10):2706-15.

[94] Kramer J, Hegert C, Guan K, Wobus AM, Muller PK, Rohwedel J. Embryonic stem cell-derived chondrogenic differentiation in vitro: activation by BMP-2 and BMP-4. Mech Dev. 2000 Apr;92(2):193-205.

[95] Richardson SM, Hoyland JA, Mobasheri R, Csaki C, Shakibaei M, Mobasheri A. Mesenchymal stem cells in regenerative medicine: opportunities and challenges for articular cartilage and intervertebral disc tissue engineering. J Cell Physiol. 2010 Jan; 222(1):23-32.

[96] Ronziere MC, Perrier E, Mallein-Gerin F, Freyria AM. Chondrogenic potential of bone marrow- and adipose tissue-derived adult human mesenchymal stem cells. Biomed Mater Eng. 2010;20(3):145-58.

[97] Hildner F, Albrecht C, Gabriel C, Redl H, van Griensven M. State of the art and future perspectives of articular cartilage regeneration: a focus on adipose-derived stem cells and platelet-derived products. J Tissue Eng Regen Med. 2011 Jan 10.

[98] Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis and rheumatism. 2005 Aug;52(8):2521-9.

[99] Yan H, Yu C. Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. Arthroscopy. [Research Support, Non-U.S. Gov't]. 2007 Feb;23(2): 178-87.

[100] Csaki C, Schneider PR, Shakibaei M. Mesenchymal stem cells as a potential pool for cartilage tissue engineering. Annals of anatomy = Anatomischer Anzeiger: official organ of the Anatomische Gesellschaft. 2008 Nov 20;190(5):395-412.

[101] Mahmoudifar N, Doran PM. Chondrogenesis and cartilage tissue engineering: the longer road to technology development. Trends in biotechnology. 2012 Mar;30(3): 166-76.

[102] Schulz RM, Bader A. Cartilage tissue engineering and bioreactor systems for the cultivation and stimulation of chondrocytes. Eur Biophys J. 2007 Apr;36(4-5):539-68.

[103] Elder BD, Athanasiou KA. Synergistic and additive effects of hydrostatic pressure and growth factors on tissue formation. PLoS One. [Research Support, N.I.H., Extramural]. 2008;3(6):e2341.

[104] Mauck RL, Nicoll SB, Seyhan SL, Ateshian GA, Hung CT. Synergistic action of growth factors and dynamic loading for articular cartilage tissue engineering. Tissue Eng. 2003 Aug;9(4):597-611.

[105] Shieh AC, Athanasiou KA. Principles of cell mechanics for cartilage tissue engineering. Ann Biomed Eng. [Review]. 2003 Jan;31(1):1-11.

[106] Waldman SD, Couto DC, Grynpas MD, Pilliar RM, Kandel RA. Multi-axial mechanical stimulation of tissue engineered cartilage: review. Eur Cell Mater. [Research Support, Non-U.S. Gov't]. 2007;13:66-73; discussion -4.

[107] Elder BD, Athanasiou KA. Hydrostatic pressure in articular cartilage tissue engineering: from chondrocytes to tissue regeneration. Tissue Eng Part B Rev. [Review]. 2009 Mar;15(1):43-53.

[108] Grad S, Eglin D, Alini M, Stoddart MJ. Physical stimulation of chondrogenic cells in vitro: a review. Clin Orthop Relat Res. [Review]. 2011 Oct;469(10):2764-72.

[109] Marlovits S, Zeller P, Singer P, Resinger C, Vecsei V. Cartilage repair: generations of autologous chondrocyte transplantation. European journal of radiology. 2006 Jan; 57(1):24-31.

[110] Harris JD, Siston RA, Pan X, Flanigan DC. Autologous chondrocyte implantation: a systematic review. J Bone Joint Surg Am. [Review]. 2010 Sep 15;92(12):2220-33.

[111] Bentley G, Biant LC, Carrington RW, Akmal M, Goldberg A, Williams AM, et al. A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. J Bone Joint Surg Br. 2003 Mar; 85(2):223-30.

[112] Harris JD, Siston RA, Brophy RH, Lattermann C, Carey JL, Flanigan DC. Failures, re-operations, and complications after autologous chondrocyte implantation--a systematic review. Osteoarthritis Cartilage. [Review]. 2011 Jul;19(7):779-91.

[113] Gigante A, Bevilacqua C, Ricevuto A, Mattioli-Belmonte M, Greco F. Membrane-seeded autologous chondrocytes: cell viability and characterization at surgery. Knee Surg Sports Traumatol Arthrosc. 2007 Jan;15(1):88-92.

[114] Bartlett W, Skinner JA, Gooding CR, Carrington RW, Flanagan AM, Briggs TW, et al. Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. J Bone Joint Surg Br. 2005 May;87(5):640-5.

[115] Basad E, Ishaque B, Bachmann G, Sturz H, Steinmeyer J. Matrix-induced autologous chondrocyte implantation versus microfracture in the treatment of cartilage defects of the knee: a 2-year randomised study. Knee Surg Sports Traumatol Arthrosc. [Randomized Controlled Trial]. 2010 Apr;18(4):519-27.

[116] Behrens P, Bitter T, Kurz B, Russlies M. Matrix-associated autologous chondrocyte transplantation/implantation (MACT/MACI)--5-year follow-up. The Knee. [Clinical Trial]. 2006 Jun;13(3):194-202.

[117] Marcacci M, Berruto M, Brocchetta D, Delcogliano A, Ghinelli D, Gobbi A, et al. Articular cartilage engineering with Hyalograft C: 3-year clinical results. Clin Orthop Relat Res. 2005 Jun(435):96-105.

[118] Pavesio A, Abatangelo G, Borrione A, Brocchetta D, Hollander AP, Kon E, et al. Hyaluronan-based scaffolds (Hyalograft C) in the treatment of knee cartilage defects: preliminary clinical findings. Novartis Foundation symposium. [Review]. 2003;249:203-17; discussion 29-33, 34-8, 39-41.

[119] Podskubka A, Povysil C, Kubes R, Sprindrich J, Sedlacek R. [Treatment of deep cartilage defects of the knee with autologous chondrocyte transplantation on a hyaluronic Acid ester scaffold (Hyalograft C)]. Acta chirurgiae orthopaedicae et traumatologiae Cechoslovaca. 2006 Aug;73(4):251-63.

[120] Kon E, Gobbi A, Filardo G, Delcogliano M, Zaffagnini S, Marcacci M. Arthroscopic second-generation autologous chondrocyte implantation compared with microfracture for chondral lesions of the knee: prospective nonrandomized study at 5 years. Am J Sports Med. [Comparative Study]. 2009 Jan;37(1):33-41.

[121] Kon E, Filardo G, Berruto M, Benazzo F, Zanon G, Della Villa S, et al. Articular cartilage treatment in high-level male soccer players: a prospective comparative study of arthroscopic second-generation autologous chondrocyte implantation versus microfracture. Am J Sports Med. 2011 Dec;39(12):2549-57.

[122] Kuroda T, Matsumoto T, Mifune Y, Fukui T, Kubo S, Matsushita T, et al. Therapeutic strategy of third-generation autologous chondrocyte implantation for osteoarthritis. Upsala journal of medical sciences. [Research Support, Non-U.S. Gov't]. 2011 May; 116(2):107-14.

[123] Hettrich CM, Crawford D, Rodeo SA. Cartilage repair: third-generation cell-based technologies--basic science, surgical techniques, clinical outcomes. Sports Med Arthrosc. [Review]. 2008 Dec;16(4):230-5.

[124] Crawford DC, DeBerardino TM, Williams RJ, 3rd. NeoCart, an autologous cartilage tissue implant, compared with microfracture for treatment of distal femoral cartilage lesions: an FDA phase-II prospective, randomized clinical trial after two years. J Bone Joint Surg Am. 2012 Jun 6;94(11):979-89.

[125] McNickle AG, Provencher MT, Cole BJ. Overview of existing cartilage repair technology. Sports Med Arthrosc. [Review]. 2008 Dec;16(4):196-201.

[126] AHCCS AsMA. AHCCCS Medical Policy Manual: Medical and Behavioral Health Policy Manual. Arizona2012.

[127] Kessler MW, Ackerman G, Dines JS, Grande D. Emerging technologies and fourth generation issues in cartilage repair. Sports Med Arthrosc. [Review]. 2008 Dec;16(4): 246-54.

[128] Steinert AF, Noth U, Tuan RS. Concepts in gene therapy for cartilage repair. Injury. 2008 Apr;39 Suppl 1:S97-113.

[129] Schiavone Panni A, Cerciello S, Vasso M. The manangement of knee cartilage defects with modified amic technique: preliminary results. Int J Immunopathol Pharmacol. 2011 Jan-Mar;24(1 Suppl 2):149-52.

[130] Gille J, Schuseil E, Wimmer J, Gellissen J, Schulz AP, Behrens P. Mid-term results of Autologous Matrix-Induced Chondrogenesis for treatment of focal cartilage defects in the knee. Knee Surg Sports Traumatol Arthrosc. 2010 Nov;18(11):1456-64.

[131] McCormick F, Yanke A, Provencher MT, Cole BJ. Minced articular cartilage--basic science, surgical technique, and clinical application. Sports Med Arthrosc. [Review]. 2008 Dec;16(4):217-20.

[132] Farr J, Cole BJ, Sherman S, Karas V. Particulated articular cartilage: CAIS and DeNovo NT. The journal of knee surgery. 2012 Mar;25(1):23-9.

[133] Cole BJ, Farr J, Winalski CS, Hosea T, Richmond J, Mandelbaum B, et al. Outcomes after a single-stage procedure for cell-based cartilage repair: a prospective clinical safety trial with 2-year follow-up. Am J Sports Med. 2011 Jun;39(6):1170-9.

[134] Rodrigues MT, Reis RL, Gomes ME. Engineering tendon and ligament tissues: present developments towards successful clinical products. Journal of tissue engineering and regenerative medicine. 2012 Apr 12.

[135] Mascarenhas R, MacDonald PB. Anterior cruciate ligament reconstruction: a look at prosthetics--past, present and possible future. McGill journal of medicine: MJM: an international forum for the advancement of medical sciences by students. 2008 Jan; 11(1):29-37.

[136] Hoffmann A, Gross G. Tendon and ligament engineering: from cell biology to in vivo application. Regenerative medicine. 2006 Jul;1(4):563-74.

[137] Sahoo S, Cho-Hong JG, Siew-Lok T. Development of hybrid polymer scaffolds for potential applications in ligament and tendon tissue engineering. Biomedical materials. 2007 Sep;2(3):169-73.

[138] Longo UG, Lamberti A, Petrillo S, Maffulli N, Denaro V. Scaffolds in tendon tissue engineering. Stem cells international. 2012;2012:517165.

[139] Thaker H, Sharma AK. Engaging stem cells for customized tendon regeneration. Stem Cells Int. 2012;2012:309187.

[140] Kuo CK, Tuan RS. Mechanoactive tenogenic differentiation of human mesenchymal stem cells. Tissue Eng Part A. 2008 Oct;14(10):1615-27.

[141] Uysal CA, Tobita M, Hyakusoku H, Mizuno H. Adipose-derived stem cells enhance primary tendon repair: Biomechanical and immunohistochemical evaluation. J Plast Reconstr Aesthet Surg. 2012 Jul 6.

[142] Kishore V, Bullock W, Sun X, Van Dyke WS, Akkus O. Tenogenic differentiation of human MSCs induced by the topography of electrochemically aligned collagen threads. Biomaterials. 2012 Mar;33(7):2137-44.

[143] Butler DL, Juncosa-Melvin N, Boivin GP, Galloway MT, Shearn JT, Gooch C, et al. Functional tissue engineering for tendon repair: A multidisciplinary strategy using mesenchymal stem cells, bioscaffolds, and mechanical stimulation. J Orthop Res. 2008 Jan;26(1):1-9.

[144] Awad HA, Boivin GP, Dressler MR, Smith FN, Young RG, Butler DL. Repair of patellar tendon injuries using a cell-collagen composite. J Orthop Res. 2003 May;21(3): 420-31.

[145] Chen JL, Yin Z, Shen WL, Chen X, Heng BC, Zou XH, et al. Efficacy of HESC-MSCs in knitted silk-collagen scaffold for tendon tissue engineering and their roles. Biomaterials. 2010 Dec;31(36):9438-51.

[146] Doroski DM, Levenston ME, Temenoff JS. Cyclic tensile culture promotes fibroblastic differentiation of marrow stromal cells encapsulated in poly(ethylene glycol)-based hydrogels. Tissue engineering Part A. 2010 Nov;16(11):3457-66.

[147] Thorfinn J, Angelidis IK, Gigliello L, Pham HM, Lindsey D, Chang J. Bioreactor optimization of tissue engineered rabbit flexor tendons in vivo. J Hand Surg Eur Vol. 2012 Feb;37(2):109-14.

[148] Woon CY, Kraus A, Raghavan SS, Pridgen BC, Megerle K, Pham H, et al. Three-dimensional-construct bioreactor conditioning in human tendon tissue engineering. Tissue Eng Part A. 2011 Oct;17(19-20):2561-72.

[149] Kajikawa Y, Morihara T, Sakamoto H, Matsuda K, Oshima Y, Yoshida A, et al. Platelet-rich plasma enhances the initial mobilization of circulation-derived cells for tendon healing. Journal of cellular physiology. 2008 Jun;215(3):837-45.

[150] Lyras DN, Kazakos K, Verettas D, Botaitis S, Agrogiannis G, Kokka A, et al. The effect of platelet-rich plasma gel in the early phase of patellar tendon healing. Archives of orthopaedic and trauma surgery. 2009 Nov;129(11):1577-82.

[151] Sanchez M, Anitua E, Azofra J, Andia I, Padilla S, Mujika I. Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. The American journal of sports medicine. 2007 Feb;35(2):245-51.

[152] de Mos M, van der Windt AE, Jahr H, van Schie HT, Weinans H, Verhaar JA, et al. Can platelet-rich plasma enhance tendon repair? A cell culture study. The American journal of sports medicine. 2008 Jun;36(6):1171-8.

[153] Virchenko O, Aspenberg P. How can one platelet injection after tendon injury lead to a stronger tendon after 4 weeks? Interplay between early regeneration and mechanical stimulation. Acta orthopaedica. 2006 Oct;77(5):806-12.

[154] Schepull T, Kvist J, Norrman H, Trinks M, Berlin G, Aspenberg P. Autologous platelets have no effect on the healing of human Achilles tendon ruptures: a randomized single-blind study. The American journal of sports medicine. 2011 Jan;39(1):38-47.

[155] Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. The American journal of sports medicine. 2006 Nov;34(11):1774-8.

[156] Kon E, Filardo G, Delcogliano M, Presti ML, Russo A, Bondi A, et al. Platelet-rich plasma: new clinical application: a pilot study for treatment of jumper's knee. Injury. 2009 Jun;40(6):598-603.

[157] Kalaci A, Cakici H, Hapa O, Yanat AN, Dogramaci Y, Sevinc TT. Treatment of plantar fasciitis using four different local injection modalities: a randomized prospective clinical trial. Journal of the American Podiatric Medical Association. 2009 Mar-Apr; 99(2):108-13.

[158] Kiter E, Celikbas E, Akkaya S, Demirkan F, Kilic BA. Comparison of injection modalities in the treatment of plantar heel pain: a randomized controlled trial. Journal of the American Podiatric Medical Association. 2006 Jul-Aug;96(4):293-6.

[159] Lee TG, Ahmad TS. Intralesional autologous blood injection compared to corticosteroid injection for treatment of chronic plantar fasciitis. A prospective, randomized, controlled trial. Foot & ankle international/American Orthopaedic Foot and Ankle Society [and] Swiss Foot and Ankle Society. 2007 Sep;28(9):984-90.

[160] de Vos RJ, van Veldhoven PL, Moen MH, Weir A, Tol JL, Maffulli N. Autologous growth factor injections in chronic tendinopathy: a systematic review. British medical bulletin. 2010;95:63-77.

[161] Vogrin M, Rupreht M, Dinevski D, Haspl M, Kuhta M, Jevsek M, et al. Effects of a platelet gel on early graft revascularization after anterior cruciate ligament reconstruction: a prospective, randomized, double-blind, clinical trial. European surgical research Europaische chirurgische Forschung Recherches chirurgicales europeennes. 2010;45(2):77-85.

[162] Vogrin M, Rupreht M, Crnjac A, Dinevski D, Krajnc Z, Recnik G. The effect of platelet-derived growth factors on knee stability after anterior cruciate ligament reconstruction: a prospective randomized clinical study. Wiener klinische Wochenschrift. 2010 May;122 Suppl 2:91-5.

[163] Nin JR, Gasque GM, Azcarate AV, Beola JD, Gonzalez MH. Has platelet-rich plasma any role in anterior cruciate ligament allograft healing? Arthroscopy: the journal of arthroscopic & related surgery: official publication of the Arthroscopy Association of North America and the International Arthroscopy Association. 2009 Nov;25(11): 1206-13.

[164] Silva A, Sampaio R. Anatomic ACL reconstruction: does the platelet-rich plasma accelerate tendon healing? Knee surgery, sports traumatology, arthroscopy: official journal of the ESSKA. 2009 Jun;17(6):676-82.

[165] Vetrano M, d'Alessandro F, Torrisi MR, Ferretti A, Vulpiani MC, Visco V. Extracorporeal shock wave therapy promotes cell proliferation and collagen synthesis of primary cultured human tenocytes. Knee surgery, sports traumatology, arthroscopy: official journal of the ESSKA. 2011 Dec;19(12):2159-68.

[166] Furia JP. High-energy extracorporeal shock wave therapy as a treatment for insertional Achilles tendinopathy. The American journal of sports medicine. 2006 May; 34(5):733-40.

[167] Costa ML, Shepstone L, Donell ST, Thomas TL. Shock wave therapy for chronic Achilles tendon pain: a randomized placebo-controlled trial. Clinical orthopaedics and related research. 2005 Nov;440:199-204.

[168] Rees JD, Maffulli N, Cook J. Management of tendinopathy. The American journal of sports medicine. 2009 Sep;37(9):1855-67.