Chapter 2

The Physiology of Sports Injuries and Repair Processes

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Additional information is available at the end of the chapter

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1. Introduction

Sports injuries are among the most common injuries and therefore present a significant public health problem. Physiologic processes after injuries are often neglected while much more attention is being paid to the management of symptoms. However, comprehension of these processes is becoming more and more important as therapies are getting increasingly focused on specific molecular and cellular processes. In recent decades, extensive research of tissue regeneration after injury and degeneration, including molecular pathways in healing, helped towards better understanding of this process and led to discoveries of new potential therapeutic targets. In this chapter physiology of sports injuries and the latest advances in understanding pathophysiological processes after injury will be discussed.

2. Physiology of tendon and ligament injury and repair

For skeletal muscles to act properly they must be attached to the bone. Tendons serve as mediators of force transmission that results in joint motion, but they also enable that the muscle belly remains at an optimal distance from the joint on which it acts. Tendons act as springs, which allows them to store and recover energy very effectively. Ligaments on the other hand attach bone to bone and therefore provide mechanical stability of the joint, guide joint motion through their normal range of motion when a tensile load is applied and prevent excessive joint displacement. Although tendons and ligaments differ in function, they share similar physiological features with a similar hierarchical structure and mechanical behavior.

2.1. Histoanatomical features of tendons and ligaments

Tendons are made up predominantly of collagen fibers embedded in proteoglycan matrix that attracts water and elastin molecules with a relatively small number of fibroblasts.

Fibroblasts are the predominant cell type in tendons. They are spindle shaped and arranged in fascicles with surrounding loose areolar tissue called peritenon. Cells are orientated in the direction of muscle loading. In mature tendon tissue they are arranged in parallel rows along the force transmitting axis of the tendon. Long cytoplasmic processes extend between the intratendinous fibroblasts, enabling cell-to-cell contact by gap-junctions.

Fibroblasts are connected to the extra cellular matrix (ECM) via integrins that permit the cells to sense and respond to mechanical stimuli which appears vital for their function because this way the mechanical continuum is established along which forces can be transmitted from the outside to the inside of the cell and vice versa. Integrins are also likely candidates for sensing tensile stress at the cell surface. It is also speculated that integrin-associated proteins are involved in signaling adaptive cellular responses upon mechanical loading of the tissue [1-5].

Type I collagen is the major constituent of tendons, accounting for about 95% of the dry tendon weight. Collagen type III accounts for about 5% of the dry tendon weight, but smaller quantities of other collagens are also present, including types V, VI, XII and type II collagen. The latter is primarily found in regions that are under compression [1-3].

Fibroblasts secrete a precursor of collagen, called procollagen, which is cleaved extracelullarly to form type I collagen. The synthesis of collagen fibrils occurs in two stages: intracellular and extracellular. The pro α-chains are initially synthesized with an additional signal peptide at the aminoterminal end with the function to direct movement of the polypeptides into the rough endoplasmic reticulum where it is cleaved off. Triple helix with three polypeptide chains wound together to form a stiff helical structure is formed intracellularly. Then the procollagen is secreted into the extracellular matrix where it is converted to collagen. Finally, collagen molecules aggregate and the cross-links responsible for its stable structure are formed [1-4].

The parallel arrangement of the collagen fibers in tendons enables them to sustain high tensile loads. Collagen molecules group together to form microfibrils, which are defined as 5 collagen molecules stacked in a quarter-stagger array. Microfibrils combine to form subfibrils, and those combine further to form fibrils (50-200 nm in diameter). Fibrils combine together to form fibers (3-7 μm in diameter) which further combine to form fascicles, and these group together to form a tendon. Fascicles are separated by endotenon and surrounded by epitenon. At the level of fascicles, the characteristic »crimp« pattern can be seen histologically (discussed later in this chapter) (Figure 1) [1-4].

Proteoglycans (PGs) account for 1-5% of the dry weight of the tendon. PGs are highly hydrophilic they attract water molecules. The predominant proteoglycans in the tendon are decorin and lumican. Biglycan and decorin (and collagen type V) regulate collagen fiber diameter in fibrillogenesis. Because decorin molecules form cross-links between collagen fibers they

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may increase the stiffness of the fibrils. Proteoglycans are also responsible for lubricating collagen fibers and thus allowing them to glide over each other [2-4]. Aggrecan, a normal structure of articular cartilage, in found in tendons that are under compression [5].

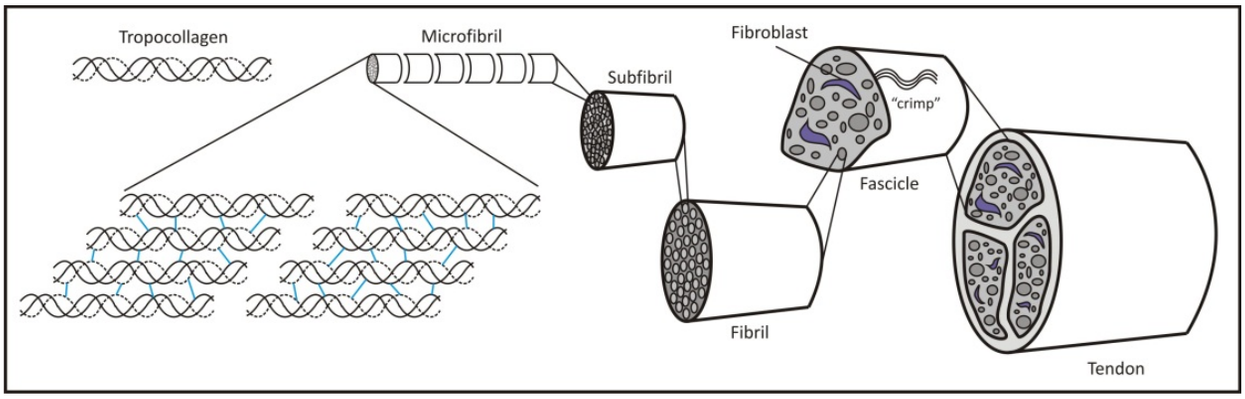

Figure 1. Structure of a tendon. See text for details. Adopted from Kastelic et al. [6]

Although tendons and ligaments are very similar in structure, there are some differences between them. (1) Ligaments consist of lower percentage of collagen molecules, but a higher percentage of the proteoglycans and water. (2) Collagen fibers are more variable and have higher elastin content and (3) fibroblasts appear rounder. (4) Furthermore, ligaments receive blood supply from insertion sites (Table 1) [1, 2].

Content / Feature	Ligaments	Tendons
Fibroblasts	20%	20%
Ground substance	20-30%	lower
Collagen	70-80%	Slightly higher
Collagen type I	90%	95-99%
Collagen type III	10%	1-5%
Elastin	Up to 2x collagen	scarce
Water	60-80%	60-80%
Organisation	More random	Organized
Orientation	Weaving pattern	Long axis orientation

Table 1. Differences between tendon and ligament structure

2.1.1. Vascular supply

There are two types of tendons: (1) tendons covered with paratenon, and (2) sheathed tendons. They mainly differ in vascular supply. In sheathed tendons a mesotenon (vincula) carries a vessel that supplies only one part of the tendon. Therefore, parts of the tendon are relatively avas-

cular and their nutrition depends on diffusion. On the other hand, paratenon-covered tendons receive their blood supply from vessels entering the tendon surface and forming a rich capillary system. Because of the difference in the vasculature, paratenon-covered tendons heal better. As stated above, ligaments receive their blood supply from insertion sites [2, 3].

There is still an ongoing debate about the efficiency of the blood supply to tendons during exercise. Experiments showed that although the increase in tendon blood flow is somehow restricted during exercise, there is no indication of any major ischemia in the tendon region. The question remains how blood flow to the tendon region is regulated. Several candidates as regulators of blood flow in skeletal muscle have been proposed, and it is possible that similar substances and metabolites are vasoactive also in the tendon region suca as bradykinin [2].

2.1.2. Insertion sites

As tendons attach skeletal muscles to bony structures, two types of tendinous junction are to be distinguished – osteotendinous where tendon attaches to the bone and musculotendinous where it attaches to the muscle. Four distinct zones have been observed at the osteotendinous junction, with a gradual change between them (Figure 2). (1) The first zone is structurally similar to the tendon propter, but with smaller amounts of PG decorin. This zone is followed by (2) fibrocartilage, where mostly collagen type II and III are found, but also small amounts of types I, IX and X. Furthermore, there is less PGs aggrecan and decorin. In the third zone, (3) mineralized fibrocartilage is made up of mainly collagen type II, but large quantities of collagen X and aggrecan are also present. The fourth zone is (4) bone, build up mainly of collagen type I and minerals [1-3].

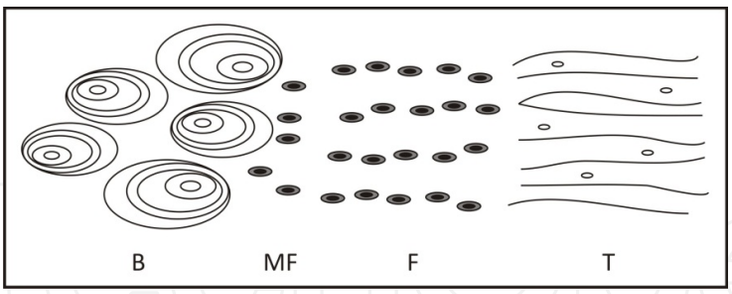

Figure 2. Diagram of a osteotendinous junction; B – bone; MF – minarelized fibrocartilage; F – fibrocartilage; T – tendon.

At musculotendinous junction, muscle cells are involuted and folded to provide maximal surface for attachment where fibrils attach. Sarcomeres of the fast contracting muscles are shortened at the junction, which may reduce the force intensity within the junction [3].

Ligaments insert into bone in two ways: through indirect or direct insertions. In indirect insertions the superficial layer is continued at with the periosteum and the deeper layer anchores to bone via Sharpey's fibers. In direct insertions, fibers attach to bone at 90° angle. Four distinct zones have been observed, with a gradual change between ligament midsubstance, fibrocartilage, mineralized fibrocartilage, and bone [2].

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2.1.3. Biomechanics of tendons and ligaments

Typical parameters describing the tendon/ligament mechanical properties are *strain*, which describes the elongation/deformation of the tendon (ΔL) relative to the normal length (L0); *stress*, the tendon force (Ft) relative to the tendon cross-sectional area (CSA), *stiffness*, the change in tendon length (ΔL) in relation to the force applied (ΔFt) and *modulus*, which describes the relation between tendon stress and tendon strain and represents the properties independently of the CSA (Figure 3 and 4). High modulus indicates stiffer tissue [7-9].

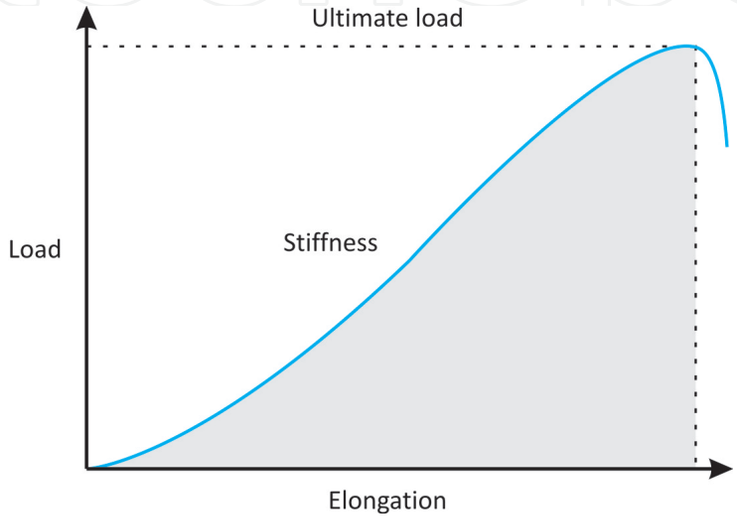

Figure 3. Structural properties of the bone-ligament-bone complex - A load/elongation curve; stiffness is represented by the slope of the curve; ultimate load is the highest load applied to the bone-ligament-bone complex before failure; the dashed area under the curve is the maximum energy stored by the complex [7, 9].

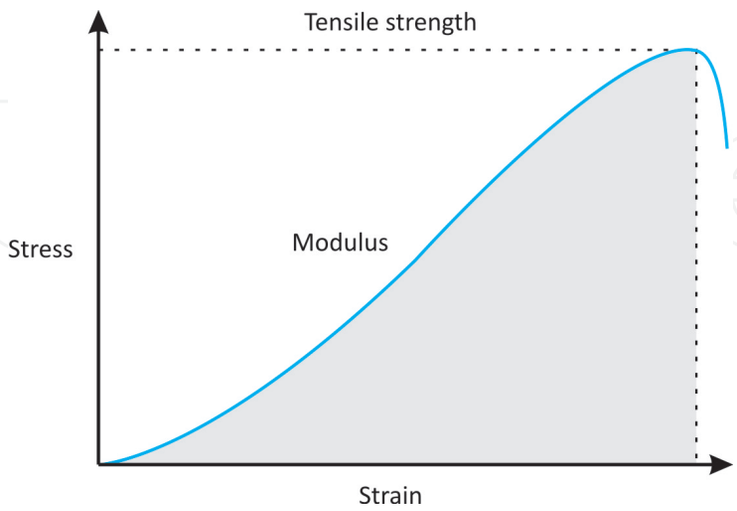

Figure 4. Mechanical properties of the bone-ligament-bone complex – A stress/strain curve; modulus is represented by the slope of the curve; tensile strength is the maximum stress of the bone-ligament-bone complex before failure; the dashed area under the curve represents the strain energy density [7, 9].

The biomechanics of ligaments is similar to tendon biomechanics. The biomechanical properties of ligaments are described as either structural properties of the bone-ligament–bone complex or the material properties of the ligament midsubstance itself. Structural properties of the bone-ligament-bone complex depend on the size and shape of the ligament, therefore they are extrinsic measures. They are obtained by loading a ligament to failure and therefore represented as a load-elongation curve between two defined limits of elongation. Mechanical properties are intrinsic measures of the quality of the tissue substance and are represented by a stress-strain curve [7, 8].

A tendon is the strongest component in the muscle-tendon-bone unit. It is estimated that tensile strength is about one-half of stainless steel (e.g. 1 cm^2 cross-section of a tendon can bear weight of 500-1000 kg) [3, 9].

2.1.4. Non-linear elasticity and viscoelasticity

There are three distinct regions of the stress/strain curve: (1) the toe region, (2) the linear region, and (3) the yield and failure region (Figure 5). In normal activity, most ligaments and tendons exist in the toe and somewhat in the linear region. This region is responsible for nonlinear stress/strain curve, because the slope of the toe region is not linear. The toe region represents "un-crimping" of the collagen fibrils. Since it is easier to stretch out the crimp of the collagen fibrils, this part of the stress strain curve shows a relatively low stiffness compared to linear portion. The toe region ends at about 2% strain when all crimpled fibers straighten. When all collagen fibrils become uncrimped, the collagen fibers stretch. The tendon deforms in a linear fashion due to the inter-molecular sliding of collagen triple helices. If strain is less than 4%, the tendon will return to its original length when unloaded, therefore this portion is elastic and reversible and the slope of the curve represents an elastic modulus. When a tendon/ligament is stretched beyond physiological limits, some fibrils begin to fail. Micro failure accumulates, stiffness is reduced and the ligament/tendon begins to fail. This occurs when intramolecular cross-links between collagen fibers fail. The tendon therefore undergoes irreversible plastic deformation. When the tendon/ligament is stretched to more than 8-10% of its original length, macroscopic failure follows [2, 3, 7].

Viscoelasticity refers to time dependent mechanical behavior. In other words, the relationship between stress and strain is not constant but depends on the time of displacement or load. There are three major characteristics of a viscoelastic material of ligaments and tendons: creep, stress relaxation, and hysteresis or energy dissipation. *Creep* indicates increasing deformation under constant load. This is in contrast with the usual elastic material, which does not elongate, no matter how long the load is applied (Figure 6). *Stress relaxation* is a feature of a ligament or tendon meaning that stress acting upon them will be eventually reduced under a constant deformation (Figure 7). When a viscoelastic material is loaded and unloaded, the unloading curve is different from the loading curve. This is called *hysteresis*. The difference between the two curves represents the amount of energy that is dissipated or lost during loading (Figure 8). If loading and unloading are repeated several times, different curves are obtained. However, after about 10 cycles, the loading and unloading curves do not change anymore, but they are still different. In other words, the amount of hysteresis un-

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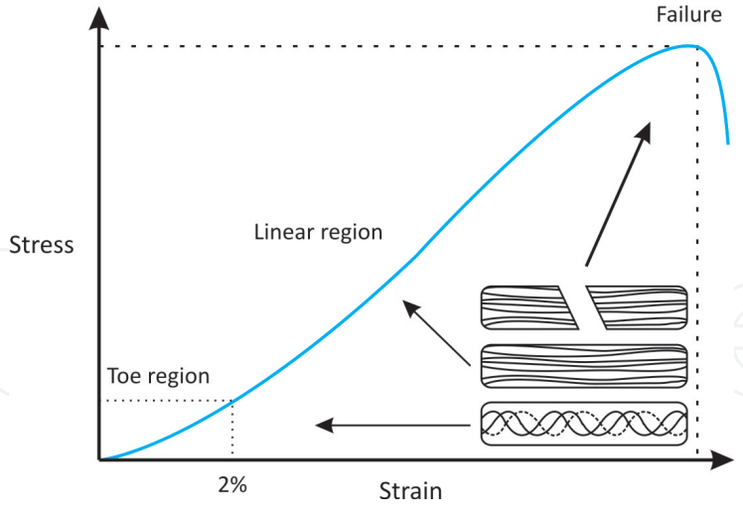

Figure 5. There are three distinct regions of the stress/strain curve: (1) the toe region, (2) the linear region, and (3) the yield and failure region. The *toe* region represents "un-crimping" of the collagen fibrils; toe region ends at about 2% of strain when all crimpled fibers straighten. It os followed by linear region, in which the collagen fibers respond linearly to load. If strain is less than 4%, the tendon will return to its original length when unloaded. Between 4 to 8 per cent of strain the collagen fibers begin to slide past one another as the cross-links start to fail which results in microscopic failure. If strain is more than 8%, macroscopic failure results.

der cyclic loading is reduced and the stress-strain curve becomes reproducible (Figure 9). This behavior is called *pseudo-elasticity* to represent the nonlinearity of ligament/tendon stress strain behavior [7].

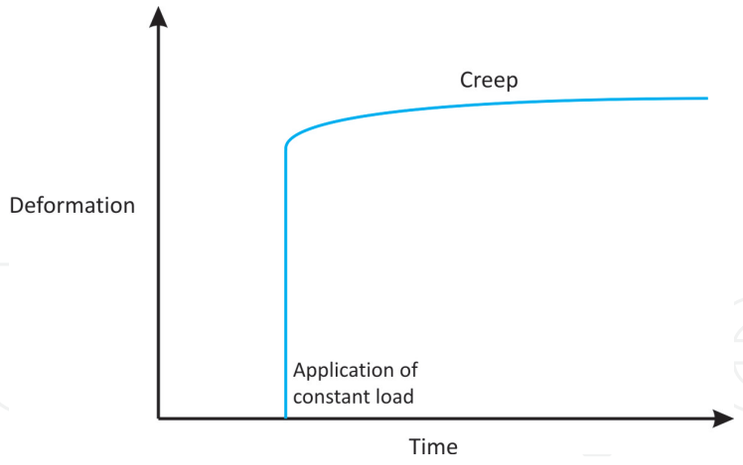

Figure 6. Creep is increasing deformation under constant load.

2.1.5. The influence of loading and gender on tendon and ligament size

Ligaments and tendons are adapted according to changes in mechanical stiffness. However, changes occur slowly, partly due to the fact that tendons and ligaments are relatively avascular tissues. There is strong evidence that tendons undergo hypertrophy, at least after long-term mechanical loading.

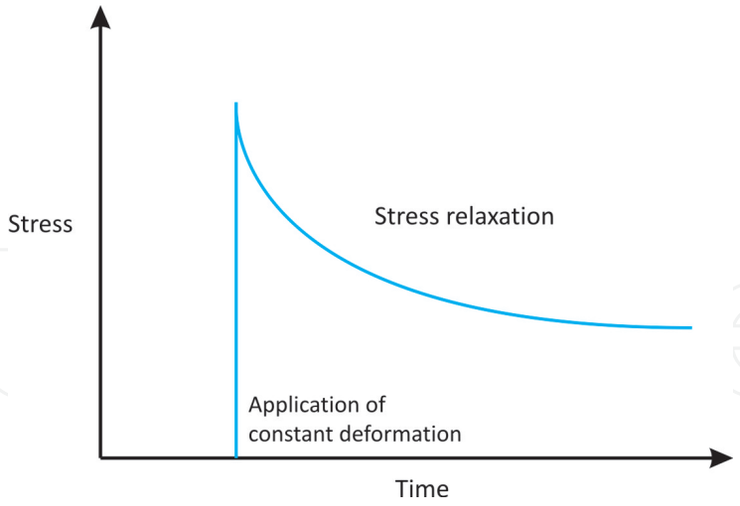

Figure 7. Stress relaxation - the stress will be reduced under a constant deformation.

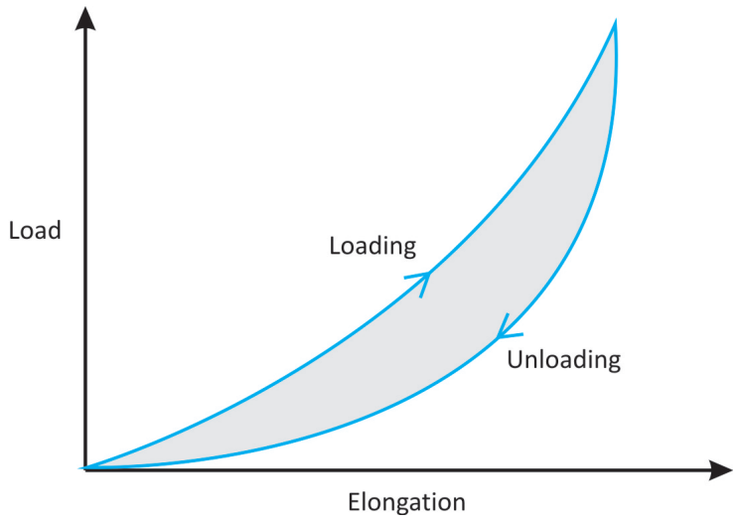

Figure 8. Hysteresis or energy dissipation – when tendon or ligament is loaded and unloaded, the unloading curve will not follow the loading curve. The energy is lost as heat (dashed area).

Male runners were found to have about larger Achilles tendon cross-sectional areas than non-runners. Furthermore, greater cross-sectional area (CSA) of patella tendons in the leading leg of male athletes competing for at least 5 years in sports with a side-to-side difference was demonstrated; an almost 30% difference in the cross-sectional area of the proximal part of the tendon between the leading and non-leading leg was observed [8, 10]. When subjected to short-term loading, only certain parts of tendons hypertrophied. It appears that tendons undergo hypertrophy in response to both long- and short-term loading, but that short-term changes in CSA are relatively small and seemingly occur only in specific regions of the tendon [8].

Interestingly, findings described above seem to be gender specific since marked differences in tendon CSA were not consistently found between female athletes and sedentary controls.

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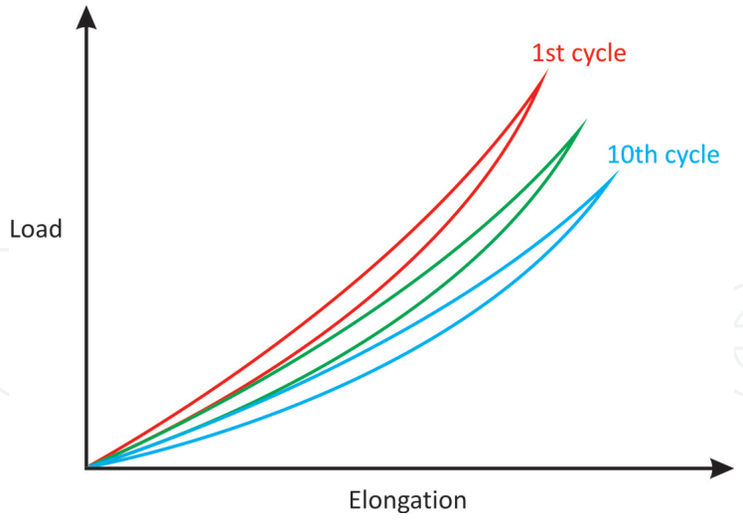

Figure 9. During cyclic loading and unloading, the stress/strain curve shifts to the right. After 10 repetitions, the curve becomes reproducible. The amount of hysteresis under cyclic loading is reduced.

Some other studies do in fact indicate that the exercise related adaptation of the tendon tissue is lower when levels of estrogen are high but the mechanism of this is not clear [8, 11]. Similary, premenopausal women were found to have lower risk for developing lower leg tendinopathies than men. The risk for developing lower leg tendinopathy in women increases in the post-menopausal period and is probably influenced by hormone-replacement therapy and activity levels. The mechanism behind this observations is not clear [12].

2.1.6. The effect of aging and immobilization ligament and tendon structure and function

With age there is an increase in the mechanical properties of ligaments and tendons up to the young adulthood when a decrease in the mechanical properties follows. Woo and colleagues tested femur-acl-tibia complex from young cadaver knees with the average age of 35 and older cadaver knees with the age of 76. They found that the linear structural stiffness of the ACL decreased both when tested at 30 degrees of knee flexion and when tested along the axis of the ligament complex [13].

Immobilization has a negative impact on tendons and ligaments [14]. Corresponding to the reduction in mechanical properties, there is a reduction in the ligament structure. Immobilization has a more rapid effect on mechanical properties than increased load from exercise. It was established that during immobilization,the cross sectional area of the ACL is reduced, which is believed to be a consequence of a loss in collagen fibrils as well as glycosaminoglycan that form the ground substance of the ligament. In addition, there might be alterations in collagen fibril orientation reducing the ligament properties. Upon remobilization, it appeared that the mechanical properties normalized first, followed by the structural properties. It is also believed that structural loss at the ligament insertion site may take longer to be removed than changes in ligament substance [7].

2.2. Tendon and ligament injury mechanisms

Tendon injury occurs because of direct trauma (i.e. penetrating, blunt, etc.) or indirect tensile overload. Acute tensile failure occurs if strain is more than 10%. However, lesser strain can cause tendon failure due to pre-existing chronic repeated insult and degeneration. Musculotendinous junction is the weakest link, especially during eccentric contractions. Maximum tension is created in forceful contractions. Furthermore, greater speed of eccentric contraction will increase the force developed. If the loading rate is slow, avulsion fracture is likely to occur. If loading is fast, tendon failure is more likely, especially if degenerated [3].

Tendon overuse injuries are a source of major concern in competitive and recreational athletes. It is estimated that 30% to 50% of all sport injuries are due to overuse [15, 16]. Studies from primary care show that 16% of general population suffers from shoulder pain, which rises to 21% in the elderly. The prevalence of Achilles tendinopathy in runners has been estimated at 11%. Tendinopathy of the forearm extensor tendons affects 1-2% of the population, most commonly occurring in the fourth and fifth decade of life. The overall prevalence of patellar tendinopathy among elite and non-elite athletes is high and varies between 3% and 45% [17]. Quadriceps tendon and tibialis posterior tendon are also often affected [15]. In the great majority of patients with spontaneous tendon rupture, the ruptured tendon shows degenerative lesions present before the rupture [16].

The term »tendinitis« has been widely used to describe a combination of tendon pain, swelling, and impaired performance. It is believed to be an inflammatory condition, although histopathological studies show degeneration rather than inflammation and therefore the term »tendinopathy« has been suggested as a more appropriate term [16, 18]. The term tendinopathy encompasses a spectrum of disorders, including lesions of the tenosynovium, the paratenon, the entesis, or tendon proper. Lesions can coexist and the tendon can tear partially or completely. Tendinopathies can be divided according to the duration of symptoms into acute (up to 2 weeks in duration), subacute (2-4 weeks), and chronic (over 6 weeks) [18].

There are multiple theories for the mechanism of tendon degeneration: (1) mechanical, (2) vascular, (3) neural, and (4) alternative theory.

In the *mechanical theory* of tendon injury, the overload of the tendon tissue is blamed for the pathologic process. Towards the higher end of the physiologic range, a microscopic failure may occur within a tendon and repetitive microtrauma can lead to matrix and cell changes, altered mechanical properties of the tendon, and symptoms development. Non-uniform stress within a tendon may produce localized fiber degeneration and damage without a history of a specific injury [15]. Studies have shown that cyclic mechanical stretching of cells can cause changes in cell morphology and alteration of both DNA and protein syntheses. In situ cell nucleus deformation does occur during tensile loading of tendons which may play a significant role in the mechanical signal transduction pathway in the affected tendon [19]. The production of prostaglandin E2 (PGE2) in tendon fibroblasts increases in a stretching magnitude-dependent manner for which cyclooxygenase (COX) is responsible [20]. Studies also showed that asymptomatic pathologic changes were common in the Achilles and patellar tendons in elite soccer players and that a greater number of hours per week resulted in a

higher prevalence of patellar tendinopathy. However, »underuse« may also be the cause of tendon degeneration because the etiopathogenic stimulus for the degenerative cascade is the catabolic response of tendon cells to mechanobiological understimulation [19].

The *vascular theory* of tendinopathy suggests that tendons generally have poor blood supply, especially the Achilles tendon and those of tibialis posterior and supraspinatus muscle. The Achilles tendon should have a hypovascular region 2-6 cm proximal to its calcaneal insertion. In such tendons overuse may lead to injury.

However, studies on the Achilles blood flow show that blood supply along the whole tendon is in fact evenly distributed throughout the tendon, but is significantly lower at the distal insertion. Blood flow in the symptomatic tendons was significantly elevated as compared with the controls, demonstrated a similar vascular response to physical loading with a progressive decline in blood flow with increasing tension [21]. Male gender, advancing age, and mechanical loading of the tendon are associated with diminished tendon blood flow [22]. Therefore, vascular theory may be more important in the lesions of fibrocartilagenous entheses that are relatively avascular, and this may contribute to a poor healing response. Angiogenesis is mediated by angiogenic factors such as vascular endothelial growth factor (VEGF). VEGF is highly expressed in degenerative Achilles tendons, whereas its expression is nearly completely downregulated in healthy tendons. Several factors are able to upregulate VEGF expression in tenocytes: hypoxia, inflammatory cytokines, and mechanical load. Since VEGF has the potential to stimulate the expression of matrix metalloproteinases and inhibit the expression of tissue inhibitors of matrix metalloproteinases (TIMP), this cytokine might play a significant role in the pathogenetic processes during degenerative tendon disease [23].

The neural theory suggests that neurally mediated mast cell degranulation could release mediators such as substance P, which is contained in primary afferent nerves. Its quantity could be related to chronic pain. The increased amount of substance P in the subacromial bursa and nerve fibers immunoreactive to substance P were localized around the vessels of rotator cuff, especially in patients with the non-perforated rotator cuff injury [24]. Inflammatory cytokines, proteinases, and cyclooxygenase enzymes, have been shown to be present in the subacromial bursa of patients with rotator cuff tear [25]. However, neural theory does not explain why morphologically pathologic tendons are not always painful [15].

The alternative theory suggests that exercise induced localized hyperthermia may be detrimental to tendon cell survival. Tendons that store energy during locomotion, such as the equine superficial flexor digitorum tendon and the human Achilles tendon, suffer a high incidence of central core degeneration which is thought to precede tendon rupture. Studies have shown that the central core of equine tendon reaches temperatures as high as 45°C during high-speed locomotion, but temperatures above 42.5°C are known to result in fibroblast death *In vitro* [26]. Temperatures experienced in the central core of the tendon *In vivo* are unlikely to result in tendon cell death, but repeated hyperthermic insults may compromise cell metabolism of matrix components, resulting in tendon central core degeneration [27].

Although exact mechanism or their combination has not been determined yet, some factors influencing the development of tendinopathy have been. There is some evidence for genetic correlation, especially with target genes close to ABO gene on chromosome 9 like COL5A1 and TNC gene [28]. Women seem to have less tendinopathy than men, especially prior to menopause. Although tendons do not degenerate with age as such, a reduction in proteoglycans and an increase in cross-links with increasing age make tendon stiffer and less capable in tolerating load. Decreased flexibility, training on harder surface, and even drugs such as corticosteroids and quinolone antibiotics have been reported to be associated with the development of tendinopathy [15].

Ligament injuries are classified into three grades. (1) Grade I injury – mild sprain. Clinically, there is minimal pain present over the injured ligament and no joint instability can be detected by clinical examination despite the microfailure of collagen fibers. (2) Grade II injury – moderate sprain or partial tear of the ligament. There is severe pain present and minimal instability detected by clinical testing. Ligament strength and stiffness decrease by 50%. (3) Grade III injury – a complete ligament tear. Most collagen fibers have ruptured and the joint is completely unstable. Another type of injury is ligament avulsion from its bony insertion. Midsubstance ruptures are more common in45 adults; avulsion injuries are more common in children. Avulsion occurs between unmineralized and mineralized fibrocartilage layers [2, 3].

2.3. Pathophysiology of tendon and ligament repair

The process of tendon healing follows a pattern similar to that of other healing tissues. There are three phases of healing: (1) hemostasis/inflammation, (2) reparative phase, and (3) remodeling and maturation phase. Ligament healing goes through the same stages as tendon healing. However, there are differences among different ligaments. A classic model for ligament healing is the rupture of medial collateral ligament of the knee (MCL). MCL has a good tendency to heal spontanelously. In contrast, the anterior cruciate ligament of the knee (ACL) does not show any tendency to heal spontaneously, which is believed to be the consequence of synovial fluid interrupting the healing process between the ruptured ends of the ligament. Therefore, an ACL reconstruction is a treatment of choice [2, 3].

After the injury, the wound site is infiltrated by inflammatory cells. Platelets aggregate at the wound and create a fibrin clot to stabilize the torn tendon edges. The clot contains cells and platelets that immediately begin to release a variety of molecules, most notably growth factors (such as platelet-derived growth factor, transforming growth factor β, and insulin-like growth factor -I and –II) causing acute local inflammation. During this inflammatory phase that usually lasts three to five days, there is an invasion of extrinsic cells such as neutrophils and macrophages which clean up necrotic debris by phagocytosis and together with intrinsic cells (such as endotenon and epitenon cells) produce a second pool of cytokines to initiate the reparative phase [2-4].

In reparative phase (three to six weeks) large amounts of disorganized collagen are deposited at the repair site with granulation tissue formation, together with neovascularization, extrinsic fibroblast migration, and intrinsic fibroblast proliferation. After four days fibroblasts infiltrate the wound site and proliferate. They produce extracellular matrix, including large amounts of collagen III and glycosaminoglycan [2-4].

In the remodeling phase, there is a decrease in the cellular and vascular content of the repairing tissue, and an increase in collagen type I content and density. Eventually, the collagen becomes more organized, properly orientated, and cross-linking with the healthy matrix outside the injury takes place. Matrix metalloproteinase degrade the collagen matrix, replacing type II collagen with type I collagen. The remodeling stage can be divided into a consolidation and maturation phase. At the end of the consolidation phase, at about 10–12 weeks, and with the beginning of the maturation phase, the fibrous tissue is converted to a stronger scar tissue. Around the fourth week collagen fibers are being longitudinally reorganized so that they are aligned in the direction of muscle loading. During the next three months the individual collagen fibers form bundles identical to the original ones. After the healing process is complete, cellularity, vascularity, and collagen makeup will return to something approximating that of the normal tendon, but the diameters and cross-linking of the collagen will often remain inferior after healing. This phase lasts for months or years, usually between 6 weeks and 9 months or more. However, the tissue continues to remodel for up to 1 year. The structural properties of the repaired tendon typically reach only two thirds of normal, even years after injury [2-4].

There are slight differences in the way different tendons heal. Extrasynovial tendons can be easily influenced by growth factors and cytokines produced by extrinsic cells (e.g. paratenon), but intrasynovial tendons are more reliant on intrinsic cells (e.g. epitenon and endotenon) [3].

2.4. Treatment of tendon and ligament injuries

According to stages of healing response, a proper rehabilitation program time frame can be introduced. During the inflammatory phase of 3-5 days rehabilitation program should avoid excess motion because it can disrupt the healing process. During the repair phase a gradual introduction of motion can be introduced to prevent excessive muscle atrophy and prevent the diminishing of range of motion (ROM). Later progressive stress can be applied, however, tendons can require up to one year to get close to normal strength levels [3, 29].

Proper postsurgical rehabilitation strategies are being debated. Rehabilitation protocols differ due to anatomical site, because different tendons have different healing characteristics. There is even a difference in the rehabilitation protocol between sheathed tendons and tendons that are not enclosed in sheaths. In sheathed tendons, early mobilization is crucial to prevent scar formation between tendon sheath, therefore diminishing ROM. The response of healing tendons to mechanical load varies depending on anatomical location. Flexor tendons require motion to prevent adhesion formation, yet excessive force results in gap formation and subsequent weakening of the repair [2, 3].

2.4.1. Immobilization and early remobilization

Ruptured and immobilized ligaments heal with a fibrous gap between the ruptured ends, whereas sutured ligaments heal without fibrous gap. The mechanical properties of scars are inferior to normal ligaments, which may lead to joint dysfunction by abnormalities in joint kinematics [30]. In spite of this, many ligaments are not repaired routinely[3].

Protective immobilization may enhance tendon-to-bone healing compared with other post repair loading regimens like exercise or complete tendon unloading. In the repaired rotator cuff, immobilization has shown to be beneficial in tendon-to-bone healing. A complete removal of loading is detrimental to rotator cuff healing. However, immobilization is not a proper treatment for all repaired tendons; some require early passive motion [4].

Tendons requiring long excursions for function (e.g. the flexor tendons) are typically encased in synovial sheaths. To maintain gliding after injury, adhesions between the tendon surface and its sheath must be prevented. Passive mechanical rehabilitation methods have shown to be beneficial to prevent fibrotic adhesions [4, 31].

The optimal time for the initiation of such treatment is about 5 days after tendon repair [31]. Controlled loading can enhance healing in most cases, but a fine balance must be reached between loads that are too low (leading to a catabolic state) or too high (leading to micro damage).

2.4.2. Surgical reconstruction

There is still a debate when ligament or tendon injuries should be treated conservatively and when surgical repair is indicated. In practice the »50% rule« is commonly used [32]. The » 50% rule« suggests that tendon/ligament injuries with structural involvement of less than 50% should be treated conservatively, but damage greater than 50% should be treated by surgical repair or reconstruction. This rule applies to a variety of orthopedic conditions, like partial fractural involvement of less than 50%, anterior cruciate ligament, partial-thickness injuries of the rotator cuff, and partial tears of the long head of the biceps tendon. However, there is very little evidence for accuracy, reproducibility, or predictive power and this rule has to be used with caution. It is maybe better to individualize the treatment according to a patient's clinical and physical status, expectations, and demands after the treatment [32].

2.5. The role of corticosteroid injection therapy

At the cellular level, anti-inflammatory and immunosuppressive actions of corticosteroids are the consequence of inhibition of cytokine-genes and pro-inflammatory mediators' synthesis, such as nitric oxide and prostaglandins. The immunosuppressive and anti-inflammatory actions of corticosteroids are mediated through the interference of two transcription factors: activating protein-1 (AP-1) and nuclear factor-κB (NF-κB) [16]. The exact mechanism by which corticosteroids inhibit the transcriptional activity of AP-1 is not fully understood. However, the activation of the cell by immune signals leads to degradation of IκB inhibitory protein from NF-κB, allowing nuclear translocation of NF-κB and consequently the transcription of multiple target genes. Corticosteroids induce the production of IκB and therefore provide efficient inactivation of NF-κB [16].

Besides the anti-inflammatory action, corticosteroids decrease the production of collagen and extracellular matrix proteins by the fibroblasts and enhance bone resorption. Furthermore, the production of extracellular matrix degrading enzymes MMP-3 (stromelysin-1), MMP-13 (collagenase-3), and MMP-1 (collagensae-1) in ligaments and other tissues is also

suppressed. Whether this is beneficial when treating chronic tendon lesions is unknown, but some reports indicate the overexpression of MMPs in the Achilles tendinopathy [16].

Corticosteroids alter mechanical properties of tendons. Incubation of tendon fibrils in corticosteroids resulted in a significant reduction in tensile strength after only 3 days [33, 34]. It is possible, that corticosteroid injection affect the component of the extracellular matrix in a way that influences tensile strength. They may reduce decorin gene expression and inhibit the proliferation and activity of tenocytes, which leads to suppression in collagen production [34]. However, the magnitude of reduction in collagen type 1 and decorin gene expression appeared to be smaller when corticosteroid treatment was combined with mechanical strain [35].

Recommendations for the use of local corticosteroid injections are still not clear. Application should be peritendinous rather than intratendinous due to the demonstrated deleterious effect of corticosteroid on tendon tissue. Short or moderate acting, more soluble preparations are recommended because in theory they cause fewer side effects (hydrocortisone, methylprednisolone). Local anesthetics are usually mixed with the corticosteroid injection for wider dispersion and more comfortable procedure; but some manufacturers warn against mixing because of theoretical risk of precipitation. Corticosteroid injections in »high strain « tendons, especially the Achilles tendon or patellar tendon, are discouraged due to the possible and well documented risk of tendon rupture [18]. This therapy should be reserved only for chronic tendon injuries after the intensive use of other approaches for at least 2 months; injections should be peritendinous only. One study showed an increased rupture risk only when corticosteroids were injected intratendionously, but not when injected in peritendinous tissue. A maximum of three injections at one site should be given with a minimum interval between injections of 6 weeks. If two injections do not provide at least 4 week's relief, they should be discontinued [18].

2.6. Future therapies to improve tendon and ligament healing

Injection of growth factors, especially those derived from activated thrombocytes, and tissue-engineering strategies, such as (1) the development of scaffold microenvironment, (2) responding cells, and (3) signaling biofactors are generating potential areas for additional prospective investigation in tendon or ligament regeneration. Tissue engendering is a promising field to enhance tendon and ligament repair. Nevertheless, significant challenges remain to accomplish a complete and functional tendon or ligament repair that will lead to a clinically effective and commercially successful application. More will be discussed in the following sections.

3. Skeletal muscle damage and repair

Musculoskeletal injuries resulting in the necrosis of muscle fibers are frequently encountered in clinical and sports medicine [36] and are the most common cause of severe long-term pain and physical disability, affecting hundreds of millions of people around the world and accounting for the majority of all sport-related injuries [37].

The annual direct and indirect costs for musculoskeletal conditions in the United States were estimated at USD $849 billion or ~ 8% of the gross domestic product. Similarly, a study published in 2009 by Fit for Work Europe, examining musculoskeletal disorders in 23 European countries, reported that > 44 million members of the European Union workforce had a long-standing health problem or disability that affected their ability to work and that musculoskeletal disorders accounted for a higher proportion of sickness absence from work than any other health condition. In 2009, the total cost of musculoskeletal disorders in European workforce was estimated at €240 billion a year [38, 39].

Injured skeletal muscle can undergo repair spontaneously via regeneration; however, this process often is incomplete because the overgrowth of extracellular matrix and the deposition of collagen lead to significant fibrous scarring [40, 41].

Muscle injuries therefore frequently result in significant morbidity, including early functional and structural deficits, contraction injury, muscle atrophy, contracture, and pain.

By neutralizing pro-fibrotic processes in injured skeletal muscle, it is possible to prevent fibrosis and enhance muscle regeneration, thereby improving the functional recovery of the injured muscle [40].

3.1. Muscle structure and mechanism of action

A number of non-contractile connective tissue elements are necessary for the organization of the contractile muscle fibers into effective mechanical stress. Thus the fibers are bound together into fascicles by the fibroelastic perimysium; the ends of the muscle are attached to the bones by tendons and aponeuroses, and the whole muscle is held in its proper place by the connective tissue sheets called fasciae [42].

The arrangement of muscle fascicles, and the manner in which they approach the tendons, has many variations. In some muscles, the fascicles are parallel with the longitudinal axis and terminate at either end in flat tendons. In case of the converging fascicles to one side of a tendon the muscle is called *penniform,* like the semimembranosus muscle. If muscles converge to both sides of a tendon, they are called *bipenniform*, or if they converge to several tendons, they are called *multipenniform*, as in case of deltoid muscle. The nomenclature of striated muscle is based on different parameters describing their properties (Table 2).

The arrangement of fascicles and the power of muscles are positively correlated. Those with comparatively few fascicles, extending the length of the muscle, have a greater range of motion but not as much power. Penniform muscles, with a large number of fascicles distributed along their tendons, have a greater power but a smaller range of motion (ROM).

Molecular basis of muscle contraction is in the interaction between *actin* and *myosin*, fuelled by ATP and initiated by the increase in $[Ca^{2+}]_i$. Skeletal muscle possesses an array of transverse T-tubules extending into the cell from the plasma membrane, through which the action potential is spread into the inner portion of the muscle fiber (Figure 10), followed by releasing a short puff of Ca^{2+} from the sarcoplasmic reticulum (SR) into the sarcoplasm. Ca^{2+} binds to troponin, a protein that normally blocks the interaction between actin and myosin. When Ca^{2+} binds, troponin moves out of the way and allows the contractile machinery to operate.

Muscle, named by	Muscle
location	• brachialis • supraspinatus
direction	• rectus abdominis • obliquus abdominis
action	• flexor hallucis • extensor digitorum
shape	• deltoideus • trapezius
attachment points	• sternocleidomastoideus • omohyoideus

Table 2. Muscle nomenclature according to different parameters.

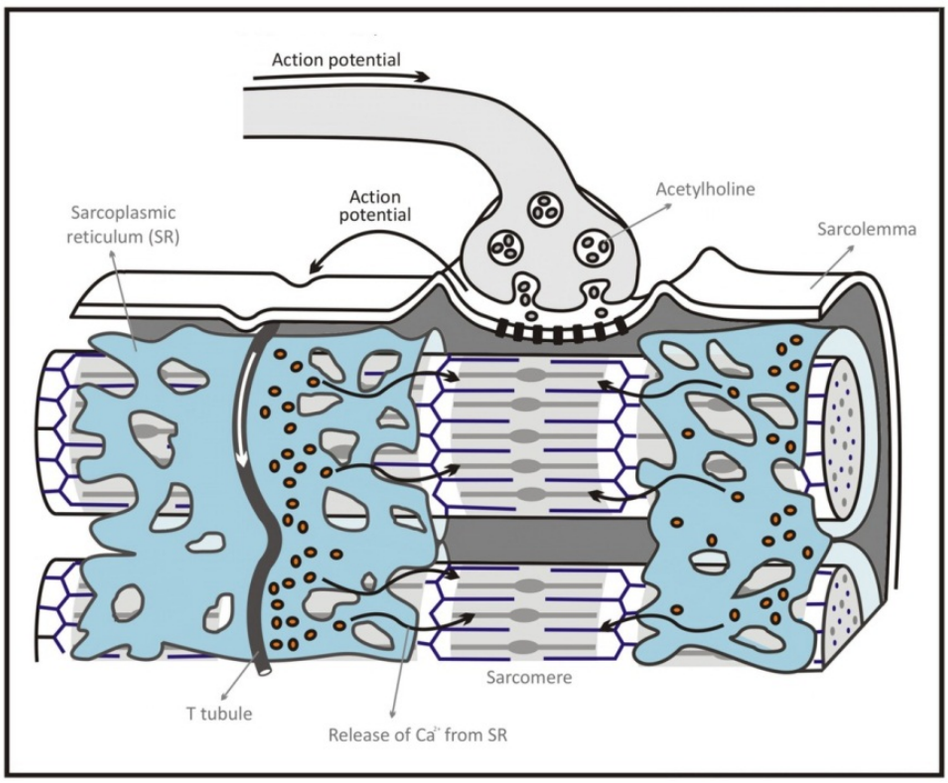

Figure 10. Molecular basis of muscle contraction.

3.2. Muscular injury mechanisms

Muscle injuries can be a consequence of a variety of causes: during the exercise, on the sports field, in the workplace, during surgical procedures, or in any kind of accidents. Regarding the mechanism, they are classified as direct and indirect. Direct injuries in-

clude lacerations and contusions, whereas the indirect class involves complete or incomplete muscle strain [43].

The current classification of muscle injuries distinguishes mild injuries from moderate and severe, based on the clinical symptoms. In a mild muscle injury, a strain or contusion is characterized by a tear of only a few muscle fibers with minor swelling and discomfort accompanied with no or only minimal loss of strength and restriction of movement. Moderate injury is represented by greater muscle damage with a clear loss of function, whereas a tear across the entire cross-section of the muscle resulting in a virtually complete loss of muscle function, is termed a severe injury [44, 45].

Muscle strain injuries after eccentric contractions are the most common type of muscle injury in athletes and are especially common in sports that require sprinting or jumping [46]. Submaximal lengthening contractions are used in everyday life, but it is well known that high-force lengthening contractions are associated with muscle damage and pain [47, 48]. Muscle strains are divided into three grades according to severity (Table 3) [43].

Muscle strains classification according to clinical severity	
Grade	**Clinical Manifestation**
I	Tear of new muscle fibers with minimal swelling and discomfort Minimal loss of strength with almost no limitation of movements
II	A greater damage of muscle Partial loss of strength and limitation of movements
III	A severe tear across the whole section of the muscle Total loss of the muscle function

Table 3. Classification of muscle strains according to clinical manifestation [43].

3.3. Pathophysiology of muscle damage and repair

The cellular and molecular mechanisms of muscle regeneration after injury and degeneration have been described extensively in recent decades [39, 49, 50]. Physiologically, healing progresses over a series of overlapping phases [43]. These stages include: (a) hemostasis, which usually starts with the formation of a blood clot and is followed by the local degranulation of platelets, which release several granule constituents; (b) the acute inflammatory phase is characterized by peripheral muscle fiber contraction, formation of edema and cell damagen and death; and (c) the remodeling phase that lasts from 48 hrs up to 6 wks; anatomic structures are restored and tissue regeneration occurs. Several cell types are involved in this phase and fibroblasts start to synthesize scar tissue.

Only local necrosis affects the injured ends of the myofibres because the torn sarcolemma is rapidly resealed, allowing the rest of the ruptured myofibres to survive [51]. Debris is removed by macrophages that secrete growth factors and activate the satellite cells. These are regenerative mononucleated stem cells of muscle tissue that normally lie between the basal

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lamina and plasma membrane of the muscle fiber [52]. First, they form myoblasts which then begin to produce muscle specific proteins and finally mature into muscle fibers with peripherally located nuclei [49].

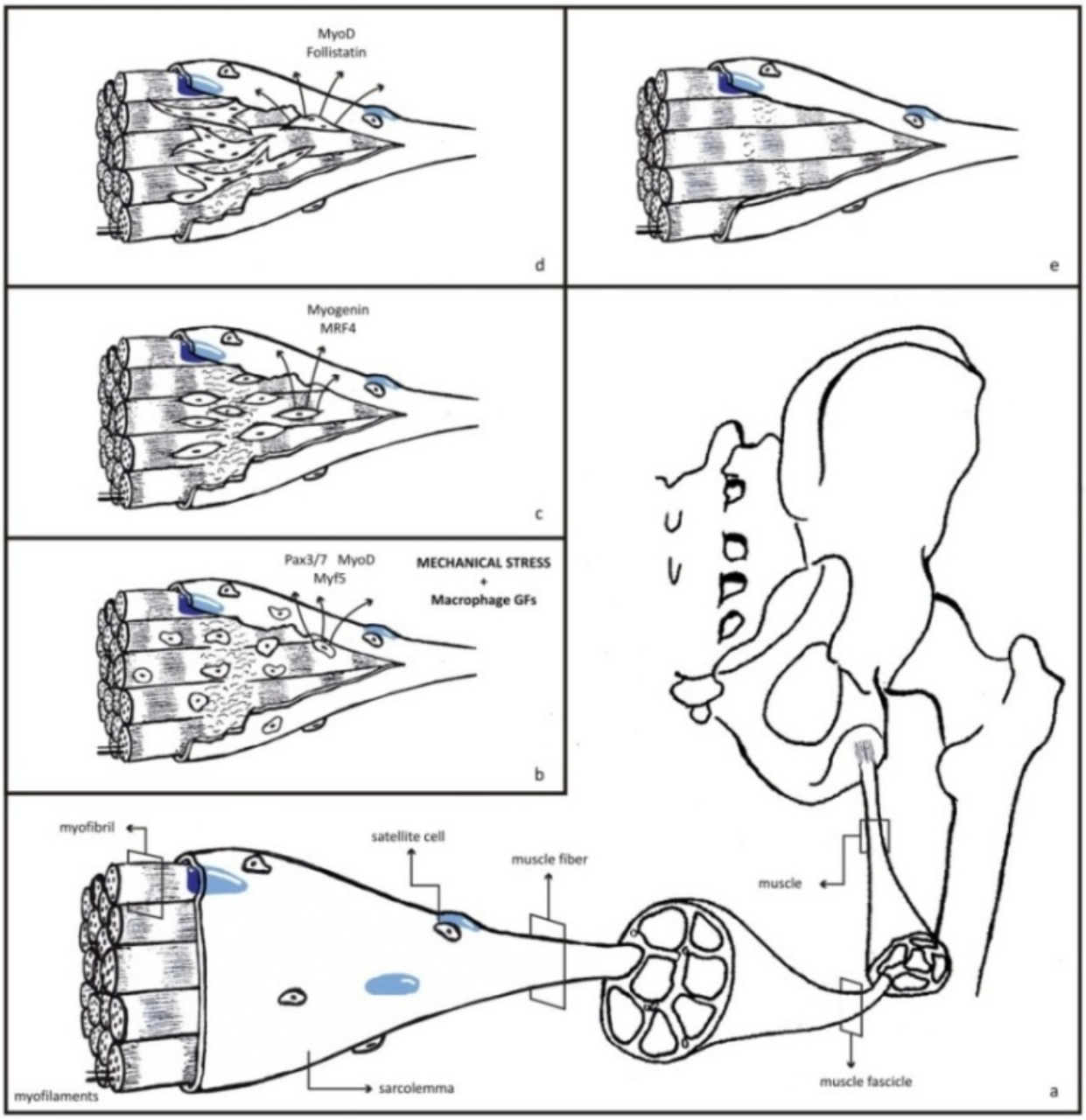

Figure 11. Role of satellite cells in muscle regeneration after acute injury. (a) quiescent satellite cells in a normal muscle just above sarcolemma; (b) mechanical stress and growth factors released from macrophages activate satellite cells that begin to express myogenic proteins which further stimulate proliferation; (c) in early differentiation phase, myoblasts express myogenin and MRF4, factors that promote further differentiation and the fusion of mononucleated cells; (d) in the late differentiation phase polynucleated myotubes begin to express factors that promote the final fusion and definite differentiation of myotubes into mature myofibres; (e) although muscle tissue is capable of self-regeneration, partial fibrosis contributes to function loss.

A typical feature during muscle differentiation is the variation in expression of various genes along with myogenic factors [53]. Sequence-specific myogenic regulatory factors (MRFs) are expressed exclusively in skeletal muscle and regulate the process of muscle development [54] (Figure 11). It is their role to govern the expression of multiple genes in myogenesis, from the engagement of mesodermal cells in the muscle lineage, to the differentiation of somatic cells and the terminal differentiation of myocytes into myofibres [55].

The MRFs consist of a group of transcription factors. They have been divided into two functional groups: The primary MRFs, MyoD, and Myf-5 required for the determination of skeletal myoblasts; and the secondary MRFs, myogenin and MRF4 that act later in the program, most likely as differentiation factors [54]. Activated satellite cells first express either Myf-5 or MyoD followed soon by co-expression of Myf-5 and MyoD. After the proliferation, myogenin and MRF4 are expressed in cells and begin their differentiation program [53].

The cellular process required for degeneration and regeneration may be affected by alterations in the inflammatory response. Although strained skeletal muscle is capable of self-regeneration, the healing process is slow and often incomplete, resulting in strength loss and a high rate of reinjury at the site of the initial injury [40]. Unfortunately, the muscle repair process involves a complex balance between muscle fiber regeneration and scar-tissue formation [39].

3.4. TGF-β and myostatin – a key factors in muscular scarring

TGF-β is a cytokine with numerous biologic activities related to wound-healing, including fibroblast and macrophage recruitment, stimulation of collagen production, downregulation of proteinase activity, and increases in metalloproteinase inhibitor activity. There are three mammalian isoforms of TGF-β: TGF-β1, TGF-β2, and TGF-β3. All three isoforms are potentially produced by most cells active in wound-healing, with platelets being a major contributor [56]. The major functions of TGF-β are listed in Table 4.

Activity of TGF-β
Stimulation of mesenchymal cell proliferation
Regulation of endothelial cells and fibroblasts
Promotion of extracellular matrix production
Stimulation of endothelial chemotaxis and angiogenesis
Inhibition of macrophage and lymphocyte proliferation
Inhibition of satellite cell differentiation

Table 4. Activity of TGF-β summarized by Borrione et al. [43]

TGF-β is a potent stimulator of fibrosis in the kidneys, liver, heart, and lungs [57-59] and is closely associated with skeletal muscle fibrosis as well where it plays a significant role in both the initiation of fibrosis and the induction of myofibroblastic differentiation of myogen-

ic cells in injured skeletal muscle [41]. Many reports indicate that the overproduction of transforming growth factor TGF-β1 in response to injury and disease is a major cause of tissue fibrosis both in animals and humans [36, 57].

Muscle-derived stem cells (MDSDs) are populations of stem cells that appear to be distinct from satellite cells and can differentiate into myofibroblasts after muscle injury [41]. But myoblasts can also differentiate into fibrotic cells where TGF-β is a key factor that stimulates fibrotic differentiation [36].

Inhibition of TGF-β has been shown to decrease collagen deposition and scarring. For example, the application of neutralizing antibodies to TGF-β in rat incisional wounds successfully reduced cutaneous scarring [53].

However, it is not yet clear whether TGF-β acts alone or requires an interaction with other molecules during the development of muscle fibrosis. Recent studies have shown that myostatin may also be involved in fibrosis formation within skeletal muscle [60, 61].

Over the last years, the TGF-β member myostatin (MSTN) has gained particular relevance because of its ability to exert a profound effect on muscle metabolism, by regulating the myofibre size in response to physiological or pathological conditions [62]. Myostatin or GDF8 (Growth differentiation factor 8) is a TGF-β protein family member that inhibits muscle differentiation and growth [63] and is expressed specifically in developing and adult skeletal muscle [62]. It inhibits the activity of satellite cells during muscle regeneration due to its control of the movement of macrophages, and also inhibits the multiplication of myoblasts and their differentiation [64]. In myogenic cells, myostatin induces down-regulation of MyoD, an early marker of muscle differentiation, and decreases the expression of Pax-3 and Myf-5, which encode transcriptional regulators of myogenic cell proliferation [65]. Its expression is restricted initially to the myotome compartment of developing somites and continues to be limited to the myogenic lineage at later stages of the development and in adult animals [53]. Major functions of myostatin are summarized in Table 5.

Activity of myostatin
Inhibition of satellite cell activity
Control of macrophage movement
Down-regulation of MyoD
Inhibition of transcriptional regulators of proliferation
Inhibition of myoblast multiplication in differentiation
Regulation of myofibre size

Table 5. Activity of myostatin.

Myostatin loss-of-function due to naturally occurring mutations into its gene triggers muscle mass increase in cattle [66], dogs [67], and humans as well [68]. Jarvnien et al. reported that the injection of a neutralizing monoclonal antibody to myostatin led to increased skele-

tal muscle mass in mice without side effects [51]. This method was found to be safe in a subsequent clinical trial, although dose escalation was limited by cutaneous hypersensitivity restricting potential efficacy [69]. Blocking of the MSTN signaling transduction pathway by specific inhibitors and genetic manipulations has been shown to result in a dramatic increase of skeletal muscle mass [70]. In principle, blocking of MSTN signaling can be achieved by three different pharmacological strategies: blocking MSTN gene expression (knocking out, inactivating the MSTN gene by viral-based gene overexpression, and antisense technologies); blocking the synthesis of the MSTN protein; and blocking of the MSTN receptor (small molecules, specific blocking antibodies) [71].

3.5. Therapeutic standards and controversies in treatment of muscle injuries

Despite the clinical significance of muscle injuries, the current treatment principles for injured skeletal muscle lack a firm scientific basis and are based on performing RICE (Rest, Ice, Compression, and Elevation). These four methods are supposed to limit the hematoma formation, though there are no randomized studies confirming their true value in the management of soft tissue injuries [72].

The most convincing is the effect of "rest" on muscle regeneration [73]. Limb immobilization prevents further retraction of the injured muscle and thereby greater discontinuity of the tissue, enlargement of hematoma, and the consequential scar tissue formation. Putting „ice"also limits the formation of the hematoma, additionally impairs inflammation, and accelerates early tissue regeneration [74]. Concerns about the limited perfusion in the damaged muscle because of the limb „compression" are putting it under question while its „elevation" above the level of the heart follows the basic physiological principles as the hydrostatic pressure in the elevated tissue falls, followed by lesser interstitial fluid accumulation and the formation of edema. In this phase it is recommended to maintain the cardiovascular fitness without the risk for reinjury like cycling or swimming [51].

Although lacking scientific background, therapeutic ultrasound is a widely accepted adjuvant method for treating muscle injuries [75]. Micro massage with high-frequency waves has a pain relieving effect and it is supposed to act proregeneratory, especially in the early phase after an injury [51]. Despite promoting proliferation, therapeutic ultrasound does not seem to have a positive effect on the final outcome of muscle healing [76, 77].

Another adjuvant therapeutic option for improving muscle repair is hyperbaric oxygen therapy (HBO), which has shown to have positive effects during the early phase of repair by accelerating the recovery of the injured muscle [78]. However, not a single randomized prospective study has been performed on the treatment of severe skeletal muscle injuries by HBO, which might increase the sensation of pain in less severe forms of injuries like delayed onset muscle soreness (DOMS) [79]. In case of both mild and severe muscle injuries there is a lack of clinical studies confirming the real place of this therapeutic option in athletes.

The use of non-steroidal anti-inflammatory drugs (NSAID's) in the treatment of muscle injuries is common, but controversial. The most commonly prescribed are COX-2 inhibitors administered either via intramuscular, oral or transdermal route [39]. While the first studies

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reported on the positive effects of NSAID's on muscle regeneration without compromising muscle contractility or stem cell proliferation, the more recent showed the importance of the inflammatory process after injury and by inhibiting it the NSAID's promote scar tissue formation [80, 81]. Incomplete muscle fiber regeneration and fibrotic infiltration can lead to long-term functional deficits and physical incapacitation [39]. The use of glucocorticoids in case of muscle injuries is even more questionable as the elimination of the hematoma and necrotic tissue seems to be slower and biomechanical strength of the injured muscle reduced [66, 82].

The identification of MRFs allows researchers a new and more detailed insight into the processes of muscle regeneration which is crucial for developing novel therapeutic targets. In recent years many studies using antifibrotic agents have been performed in patients with different heart and kidney diseases or systemic sclerosis. *In vitro* and *In vivo* studies showed important antifibrotic effects of platelet-rich plasma derived growth factors, recombinant proteins such as decorin, follistatin, γ-interferon, suramin, relaxin, and other biologically active agents like mannose-6-phosphate, N-acetylcysteine, and angiotensin-receptor blockers. Although none of these has yet been tested on humans, their promising effects may significantly alter the therapeutic options of muscle injuries in the future. Furhter discussion on these bioactive agents will follow in Chapter XX (numer needed: Latest advances).

4. Articular cartilage damage and repair

Cartilage comprises of inherited limited healing potential and thus remains a challenging tissue to repair and reconstruct. Traumatic and degenerative cartilage defects occur frequently in the knee joint and represent difficult clinical dilemma. Articular cartilage has a limited capacity to self-repair principally due to its avascular nature and the limited ability of mature chondrocytes to produce a sufficient amount of extracellular matrix. Untreated cartilage injuries therefore lead to the development of arthritis. Current first line treatment options for smaller and mid-sized lesions in lower-demand patients are debridement or lavage and bone marrow-stimulating techniques (microfracture) which promote a fibrocartilage healing response. On the other hand, restorative treatment options such as osteochondral autologous graft transplantation (OATS) are limited by the amount of donor tissue availability and the size and depth of the defect. Regenerative treatment techniques such as autologous chondrocyte implantation (ACI) are promising treatment options for large full thickness articular cartilage defects where cells from healthy non-weight bearing areas are multiplied *In vitro* and implanted into such defects. Opposed to the traditional reparative procedures (e.g. bone marrow stimulation – microfracture), which promote a fibrocartilage formation with lower tissue biomechanical properties and poorer clinical results, ACI is capable to restore hyaline-like cartilage tissue in damaged articular surfaces. This technique has undergone several advances and is constantly improving. Indeed, there are numerous studies exploring new biomaterials; applications of various growth factors; the synergistic effects of mechanical stimulation in terms of tissue engineering *In vitro*, *In vivo*, and in animal models in order to stimulate the formation of hyaline-like cartilage.

4.1. Cartilage structure

Articular (hyaline) cartilage is a specific and well-characterized tissue with remarkable mechanical properties consisting of exclusively one cell type - chondrocytes which are embedded in the extracellular matrix (ECM). The principal function of articular cartilage is to withstand mechanical loads, facilitate smooth and perfect glide among articular surfaces, and enable painless and low friction movements of synovial joints. The articular cartilage is an aneural, avascular and alymphatic structure. The nutrition of chondrocytes occurs via diffusion between synovial fluid and cartilage matrix.

The only resident cells in articular cartilage (chondrocytes) contribute to only 1-5 % of tissue volume. The remaining 99 % represent the extracellular matrix (ECM) structural components that mainly consist of water, collagen, and proteoglycans (PGs). ECM works as a biphasic structure composed of a fluid phase (water and electrolytes) and solid phase consisting mainly of collagen and proteoglycans. The solid phase comprises of low permeability due to the high resistance of a fluid flow which causes a high rate of fluid pressurization and contributes to the load transmission of cartilage. Together, both solid and fluid phase establish the stiffness and viscoelastic properties of a cartilage [83, 84].

4.1.1. Structural layers

The structure of cartilage matrix varies with the depth; four different zones (superficial, transitional, radial, and calcified) are distinguished based upon the cell morphology, matrix composition, and collagen fibril orientation (Figure 12). Chondrocytes change their conformation from parallel to vertical in deep zones. Similarly, collagen fibers alignment becomes parallel in deeper zones of cartilage tissue. There is also an increase in the overall volume, water content, and overall biological activity in deeper zones [85].

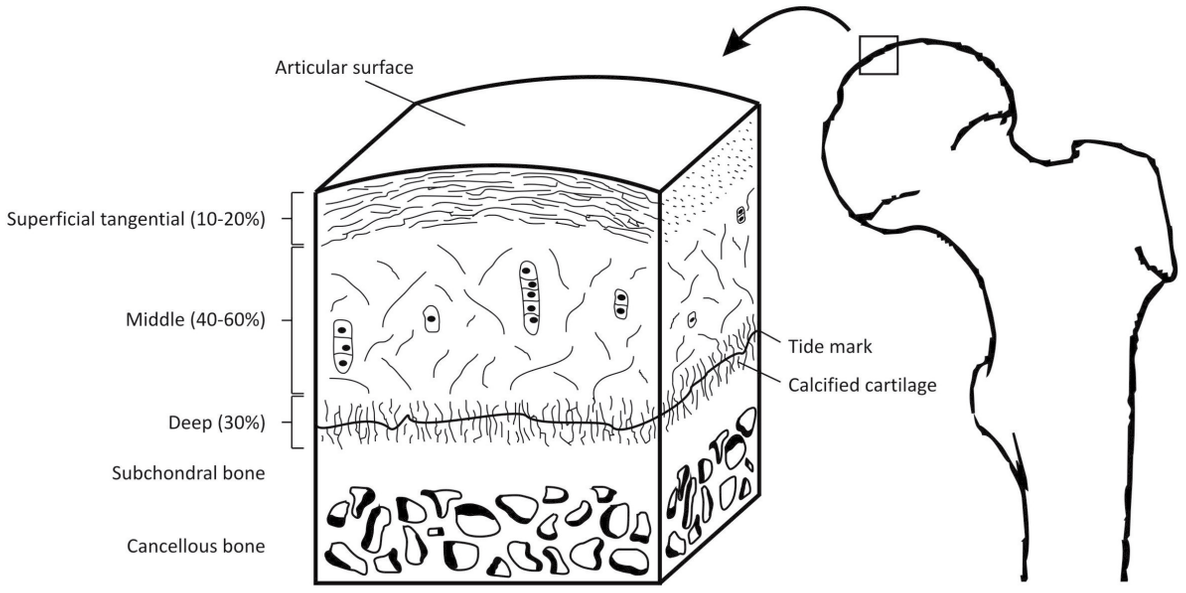

Figure 12. Structural layers of articualar cartilage.

Chondrocytes are specialized cells and basic structural cells in the articular cartilage, which are sparsely spread within the matrix and altogether form only 1-5 % of cartilage volume. They are deprived of blood supply and obtain the nutrients by diffusion from synovial fluid.

The formation of cartilage tissue and maturation of chondrocytes follows a multi-step process called chondrogenesis. In general it comprises of mesenchymal stem cell proliferation and their differentiation into mature chondrocytes capable to synthesize structural components of ECM (type II collagen, PG and non-collagenous proteins) and to maintain its continuous formation and restoration.

Each step of chondrogenesis can be classified according to the expression of different sets of transcription factors, cell adhesion molecules and extracellular matrix components. Chondrocytes have no cell-to-cell contacts, are highly metabolically active (however, due to low overall cell volume the total activity appears low) and are exposed to low oxygen environment and anaerobic metabolism. Mature chondrocytes are in the continuous communication with ECM and hence respond to changes in ECM and regulate its metabolism [85, 86].

Cartilage tissue is under constant impact of anabolic and catabolic cellular activity in response to extracellular environment and exposure to different cytokines and growth factors. Anabolic proteins such as tumor growth factor beta (TNF-beta), insulin growth factor (IGF-1), bone morphogenic protein (BMP), and fibroblast growth factor (FGF) stimulate matrix formation and promote the anabolic activity of chondrocytes. On the other hand, catabolic proteins such as tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) inhibit protein synthesis and promote matrix degeneration. The constant equilibrium in the functioning of all signaling pathways is of crucial importance for the proper function and maintenance of cartilage tissue. The modern concept of cartilage tissue engineering is based on the imitation of the cartilage natural environment and the process of chondrogenesis to try to stimulate the formation of such a cartilage, which contains all the structural and biomechanical properties of native cartilage [87].

Extracellular matrix (ECM) is consists of water, collagen, and proteoglycans. All together water represents 60-85 % of the weight of the cartilage. The water content varies with the depth of the tissue; near the articular surface the water content is the highest and PG concentration is relatively low; vice versa is found in a deeper zone near subchondral bone, where the water content is the lowest but the PG concentration is the greatest. A high amount of water content in cartilage tissue is important for nutrition, lubrication, and for creating a low-friction gliding surface. In diseased cartilage such as osteoarthritis, the water content amounts to more than 90% as a result of matrix disruption and increased permeability. This leads to the decreased modulus of elasticity and reduction in load bearing capability.

Collagen is the main component of ECM. This fibrous protein represents 60 to 70% of the dry weight of the tissue. Type II collagen is the predominant collagen (90–95%) of ECM and provides a tensile strength to the articular cartilage. The high rate of cross-linkage between collagen molecules provides cartilages its resistance against traction forces. Other types of collagen molecules are also found in cartilage tissue in smaller amounts, these are types V, VI, IX, X and XI. Type IX and XI are most abundant in minor types collagen. Type XI partici-

pates in cross-linkage with type II collagen, integrins, and proteoglycans, whereas type XI is important in regulating the fibril diameter of type II collagen. Collagen architecture varies through the depth of the tissue. On the sliding surface of entire cartilage (tangential zone) collagen fibers are oriented parallel to the cartilage surface.

Proteoglycans (PGs) are protein polysaccharides and form 10–20% dry weight of the articular cartilage. Their primary function is to provide compressive strength to cartilage tissue. In articular cartilage they can be classified in two major classes, large aggregating proteoglycan monomers (aggrecans) and small proteoglycan molecules (decorin, biglycan, and fibromodulin). PG are composed of glycosaminoglycans (GAG) subunits (chondroitin and keratin sulfate) which are bound to a central core protein via sugar bonds to form proteoglycan aggrecan, which is highly characteristic for hyaline cartilage. Aggrecan, 250 kDa protein represents more than 80 % of all PG molecules in cartilage tissue. It binds to hyaluronic acid to form high molecular weight aggregates with more than 3.5 x 106 kDa. In the cartilage tissue these aggregates are located within the collagen type II fibril network resulting in densely packed negative charge which interacts with water via hydrogen bond and causing electrostatic repulsion. This key feature enables cartilage tissue to resist deformation under compression and to withstand and redistribute mechanical [83] [84].

4.2. Cartilage lesions

Injuries to articular cartilage are observed with an increasing frequency in athletes. In particular participation in pivoting sports such as football, basketball, and soccer they are associated with a rising number of sport-related cartilage injuries. The exact incidence of the cartilage damage is not known since they mostly appear asymptomaticly. However, during a review of 25,124 and 31.516 knee arthroscopies the injury of articular cartilage was found in 60 - 63 % [88, 89]. The incidence of 5 – 11 % was reported for full-thickness cartilage lesions (ICRS grade III and IV) [90]. Additionally, cartilage injuries of the knee joint are often accompanied with other acute injures such as ligament and meniscal injuries, traumatic patellar dislocation, osteochondral injuries, etc. [91].

The main symptom in patients with cartilage defects is the joint pain. Patients may also experience swelling and mechanical symptoms. Traumatic cartilage injury in the athletic population may progress to chronic pathological loading patterns such as joint instability and axis deviation. Although intact articular cartilage has the ability to adjust to the increasing weight bearing activity in athletes by increasing cartilage volume and thickness recent studies indicated that the degree of adaptation is limited [92]. Any activity beyond a threshold value may therefore result in maladaptation and cartilage damage. It has been shown that high impact joint loading above the adaptation limit causes decreased PGs content and leads to increase of degradative enzymes release and chondrocytes apoptosis [93]. Eventually, the integrity of functional weight bearing unit of cartilage is disrupted and leads to the loss of articular cartilage volume and stiffness, elevation of pressure and further articular cartilage damage in the long run.

Clinically, focal lesions are ranked according to the appearance of superficial zone of articular cartilage and are generally small (<1cm2) and sub-chondral and therefore asymptomatic.

It is difficult to predict whether the chondral lesion will progress to the more extensive degradation. However, in animal studies it was observed that smaller defects have the potential of spontaneous healing while the inverse relationship to repair potential was revealed in larger defects [94]. Once a patient becomes symptomatic due to cartilage damage, the lesion is likely to progress. A mechanical injury to articular cartilage can be acute, chronic, or acute and chronic. Cartilage loss often occurs after single or repeated impact loading due to trauma or misalignment. An increase in shear forces as a consequence of chronic abnormal loading of a joint surface results in irreversible changes in the biochemical composition of articular cartilage. Loading studies reported of significant swelling of articular cartilage (increased water content) and changes in the proteoglycans content only two weeks after abnormal loading [95].

Cartilage tissue has a limited intrinsic capacity of healing response after cartilage damage, thus cannot fully regenerate and often leads to secondary degenerative disease. Early recognized and treated cartilage lesions might therefore prevent the secondary damage and progression to the osteoarthritis. The main raisons for limited capacity to self-repair and regeneration seem to be the avascular nature of cartilage tissue and inability for clot formation, which is the basic step in the healing cascade. That is why progenitor cells in blood and bone marrow and resident chondrocytes are unable to migrate to sites of the cartilage lesion [96]. Generally, intrinsic cartilage repair does not follow the main steps that usually occur after an injury in the other tissue: necrosis, inflammation, and repair or remodeling. Furthermore, mature chondrocytes own limited proliferative capacity and have the limited ability to produce a sufficient amount of extracellular matrix to cover the defect. However, several cells are mobilized to the cartilage surfaces after an injury and can produce the repair matrix, although this matrix is morphologically and mechanically inferior to the original native cartilage tissue. Such a spontaneous healing was observed in small sub-chondral defects of fetal lambs and partial healing was also detected in small (less than 3 mm diameter) full-thickness lesions in rabbits [97]. However, larger cartilage defects of more than 6 mm rarely, if ever, show intrinsic healing potential but lead to progressive degenerative disease [94].

4.2.1. Partial and full thickness defects

Cartilage lesions can be divided into partial thickness defects which do not penetrate the subchondral bone and do not repair spontaneously, and full thickness defects which do penetrate subchondral bone have a partial repair potential, depending on the size and locations of the defect (Figure 13) [98]. The nature of the partial thickness defects has been studied and it was observed that the cells adjacent to the wound margin undergo cell death. However, there is an increase in cell proliferation, chondrocyte cluster formation, and matrix synthesis, but this repair is short-lived and eventually fails to repair the defect. It was also documented that the cells from synovia can migrate to the lesion in the presence of growth factors and can fill the defect with repair tissue. Due to anti-adhesive properties of PG and the absence of fibrin matrix these cells usually fail to adhere to the surface of defect [99].

The potential of cartilage repair in full thickness lesions is due to breaching of subchondral bony plate which leads to local influx of blood and undifferentiated mesencyhmal cells and

hematoma formation containing fibrin clot, platelet, red and white blood cells. The blood clot can only fill the smaller defects < 2-3mm in diameter from the subchondral bone marrow. However, mobilized cells in the newly formatted blood clot are not capable to replace the defect with native hyaline cartilage, but produce fibrocartilage tissue, composed of higher collagen type I to collagen type II ratio and less proteoglycan, which has as mentioned already inferior properties compared to native hyaline cartilage. Several surgical techniques used the same attempt to treat full thickness defects such as micfrofracture which penetrate the subchondral bone in order to stimulate the clot formation and immobilize cells to the side of cartilage lesion [98].

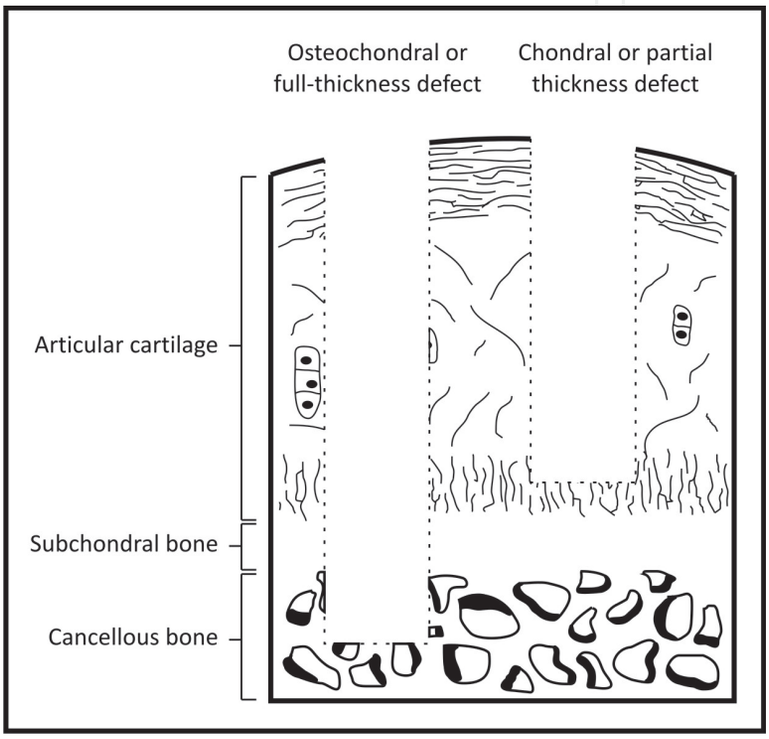

Figure 13. Partial and full thickness defects of articular cartilage.

4.2.2. Cartilage lesion classification

There are several classification systems to access cartilage lesion used in clinical practice. A number of elements are important in deciding what intervention might be the most helpful in trying to restore cartilage tissue such as: the size and area of cartilage damage, the depth of the damage, the degree of functional disability, patients' age, etc. However, not enough is known about a proper treatment of particular cartilage. Therefore, more objective data, methods and operative outcomes are required for good decision making regarding the treatment modalities since new procedures are rather expensive. Currently, the structural classifications such as Outerbridge and ICRS Classification (Table 6) are commonly used involving the examination of the extent and the depth of the cartilage lesion that helps surgeons to follow progression and improvement of the cartilage lesions.

	OUTERBRIDGE - description	ICRS - description
GRADE 0	normal cartilage	normal cartilage
GRADE 1	cartilage with softening and swelling	nearly normal: soft indentation and/or superficial fissures and cracks
GRADE 2	a partial-thickness defect (fibrillation or superficial fissures) less than 0.5-in diameter	a partial-thickness defect: extending down to <50% of cartilage depth
GRADE 3	deep fissuring of the cartilage to the level of subhondral bone without bone exposed greater than 0.5-in diameter	a partial-thickness defect: extending down to "/>50% of cartilage depth
GRADE 4	exposed subchondral bone.	severely abnormal (through the subchondral bone)

Table 6. Classification of articular cartilage lesions: Outerbridge and ICRS classification

The modified ICRS classification describes the defect macroscopically and correlates better with clinical outcome; grade 1 has good, grade 2-3 intermediate and grade 4 poor clinical result. However, along with the grade and depth, it is important to record the dimensions and position of the lesion (Modified ICRS Chondral Injury Classification System), to assess any bone loss or sclerotic change, the thickness of the surrounding cartilage and surrounding walls. Additionally, overall outcome depends also on patient's age, BMI index, the level of physical activity, etc.

4.3. Treatment of articular cartilage lesions

The main goals of surgical management of cartilage defects are to reduce symptoms, restore cartilage congruence, prevent additional cartilage deterioration, and to maintain the function of the joint without the insertion of artificial implants. Surgical treatment options may be divided upon their expected outcome as palliative, reparative or restorative [15]. Many procedures lead to the formation of fibrocartilaginous tissue with significantly inferior biochemical properties compared with those of hyaline cartilage. The newly formed scar tissue is unable to prevent a progression of a degenerative cartilage disease. The application of a specific surgical method is based on the patient's demand and the level of symptoms. For example, in lower demand patients with fewer symptoms the effective first-line treatments are palliative such as debridement and lavage. Similarly, reparative techniques are used in patients with moderate symptoms such as bone marrow stimulating procedures (drilling, abrasion arthroplasty, or microfracture) in effort to promote fibrocartilage formation. However, larger cartilage defects in higher demand patients (e.g. athletes with extreme weight bearing activity) with significant symptoms may not profit from standard treatment options, but should be advanced towards reparative treatment options such as autologous chondrocyte implantation (ACI) or osteochondral grafting [100].

4.3.1. Debridement and lavage

The goals of palliative treatment options (debridement and lavage) are the reduction of the inflammatory response due to mechanical irritation, functional improvement, and pain relief. Debridement involves the smoothing of cartilage and meniscal surfaces, removing necrotic tissue, and refreshing edges of cartilage lesions. Likewise, the beneficial effect of lavage implies the reduction of inflammation; removal of free cartilage fragments due to an injury and potential calcium phosphate crystals. Although the effectiveness of such a method is short-termed since it does not apply the restoration of cartilage defects, it significantly reduces pain symptomatic and improves the functionality of the articular joint compared to the conservative therapy. It is primarily recommended for patients with lower daily physical load and specifically localized mechanical symptoms (e.g. meniscal tear). Rehabilitation time after surgery is short and allows immediate loading activities without restrictions [101, 102].

4.3.2. Marrow stimulating techniques

Articular cartilage is deprived of its own blood supply; therefore traditional wound healing and clot formation is not possible. By opening up the subchondral bone plate, which separates the cartilage layer from the blood supply in bone marrow, hemorrhage can be induced to stimulate mesenchymal stem cells (MSCs), leukocytes, and growth on the side of the lesion as well as trigger remodeling and fibrocartilaginous cartilage repair. Bone marrow stimulating techniques are divided into drilling, microfracture, and abrasion, and are all based on the infiltration of blood products, fibrin clot formation, and fibrocartilage tissue repair [103].

Nowadays, microfracture is often used as a primary treatment option, and if not successful, more invasive cartilage repair methods are performed. The procedure is performed arthroscopically after a careful examination of articular cartilage surface and the quality of the cartilage. First, the focal chondral defects are debrided and the walls of the defect are smoothened. Any calcified cartilage is removed from the defect zone in order to prepare a better surface for the adherence of the clot and improved chondral nutrition through subchondral diffusion. Likewise, the walls of the lesion should be perpendicular to the defect to provide an area where the clot progenitor cell can form and adhere. After the initial preparation, the surgical awl is used to make multiple holes in the exposed subchondral bone. The holes should be placed 3-4mm from each other and should not connect. Subsequently, blood clot rich with bone marrow elements is formed which eventually undergoes the phase of remodeling and turns into fibrocartilage tissue [101-103]. Such cartilage resembles the native cartilage, but it differs significantly in the structural, biochemical, and mechanical properties and mostly contains type I collagen, which is cartilage non-specific and results in poor mechanical properties and poorly integrates into the adjacent cartilage.

A major concern is therefore the longevity of a fibrocartilage to withstand the stress and mechanical load on an active knee joint [104]. However, in follow-up studies 7-10 years after the surgery pain release and improved joint functionality was reported [105]. Moreover, microfracturing seems to have similar clinical results as ACI (look further chapter). Another

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problem was recently reported with microfracture procedure whether it can decrease the success of further alternative procedures such as ACI. In the study patients allocated to bone stimulating technique showed similar results following ACI as those where only debridement alone was performed [106]. Furthermore, in another study patients who previously underwent bone stimulating procedure showed a poorer outcome after ACI compared to those where only ACI alone had been performed [107].

Postoperative rehabilitation plays a key role in overall success of the treatment. Patients should undergo continuous passive motion physiotherapy for a period of 4 to 6 weeks and have the protected weight bearing. Following that period, patients are allowed an active range of motion exercises and progression to full weight bearing. However, no cutting, jumping or twisting sports are allowed until at least 4 – 6 months after surgery.

4.3.3. Osteochondral autograft transplantation (AOT)

Regeneration of damaged cartilage can be achieved with bone-cartilage transplants called osteochondral autograft transplantation (AOT). Nowadays, AOT is a well-established technique, but since the majority of the cartilage defects found in the knee joint are chondral rather than subchondral, there is a controversy regarding the overall usage of a osteochondral grafts and reaming in the healthy subchondral bone. The surgical procedure of AOT involves the removal of a full thickness hyaline cartilage attached to its underlying bone and the implantation of the osteochondral graft on the side of the lesion in a press-fit technique. Osteochondral autografts are usually harvested from non-weight bearing areas in order to avoid new damage or loss of function on the donor side. The site of the lesion should be prepared prior to implantation; any remaining cartilage is removed, the walls of the defect are made smooth and the tunnel of the same size as of the cartilage plug is drilled. However, the depth on the damage site should be 2 mm less than the plug size in order to achieve a favorable and stable position of the osteochondral graft and maintain an appropriate fit to the edges of the graft with surrounding intact cartilage. This helps to reduce shear stress on the border of the graft and ensures long-term success of the transplantation. Cartilage defects should not range more than 3cm2 due to a limited amount of donor tissue. For larger lesions several osteochondral plugs are used, therefore the procedure is called »mosaicoplasty«.

The main advantage of osteohondral grafting is that it possesses the normal native hyaline cartilage and does not include fibrocartilage which develops in the microfrature technique. However, the disadvantages include donor side morbidity (pain and cartilage defect), technical difficulty to match the shape of the plug to the contour of the articular joint, residual gap between adjacent plugs, and the risk of osteochondral collapse. Postoperative rehabilitation contains the use of continuous passive motion machine and weight bearing restrictions for a period up to 6 weeks. Clinical results are satisfactory; they reported good to excellent results even 10 years after surgery in 79 - 92% patients. The effectiveness of the method depends on the site of injury and is the most successful in isolated injuries of the femur condyles [101, 102].

4.3.4. Autologous cartilage implantation (ACI)

Autologous cartilage implantation represents a promising solution for the treatment of articular cartilage and enables permanent replacement of damaged cartilage tissue with its own native hyaline cartilage. The idea of an ACI is to harvest cartilage cells from the knee and grow them *In vitro* under specific laboratory conditions (Figure 14). Once millions of cells have been grown they are implanted into the area of cartilage defect. The procedure was first proposed by Brittberg in 1994 [108] and has become more widespread so that it currently represents the most developed articular cartilage repair technique.

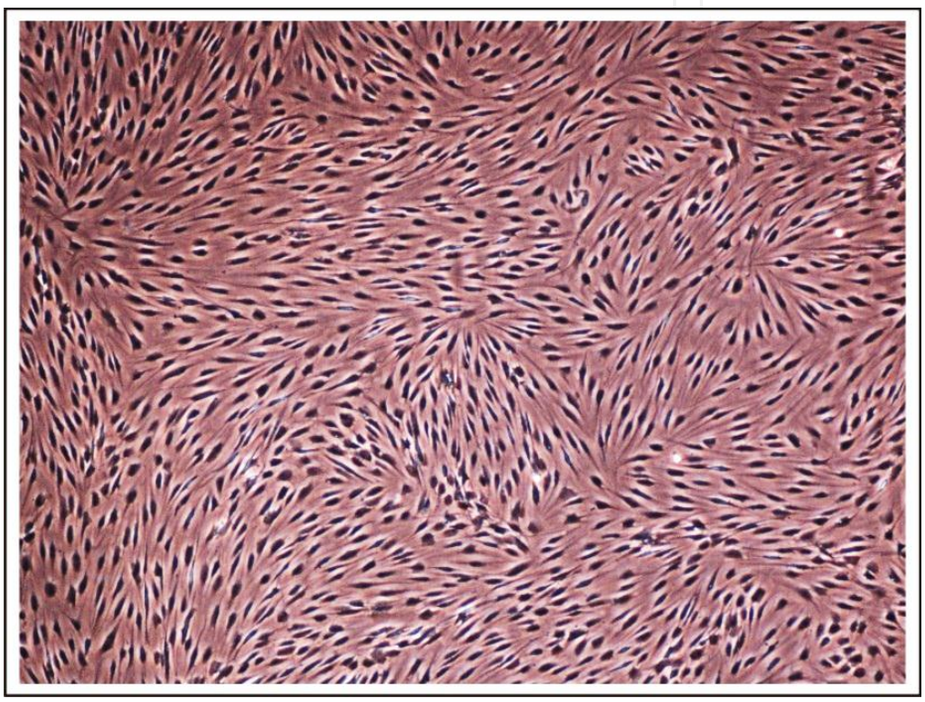

Figure 14. Proliferation of chondrocytes under monolayer culturing condition.

The original technique of ACI is a two-step procedure (Figure 15). The first step of ACI includes an arthroscopy to identify and access cartilage damage. Once the lesion is determined as suitable to perform the ACI procedure, the cartilage cells are harvested from the non-weight bearing zone in the knee. The chondrocytes are then isolated and grown in the tissue culture to allow them to multiply for several weeks. Once a sufficient number of cartilage cells has been obtained in the culture, the second surgery is scheduled. During the second surgery the cell suspension is re-injected into the cartilage defect underneath the periosteal patch. It is very important that the periosteal patch is carefully sutured in place and sealed with a fibrin glue in order to prevent any leakage of newly implanted cell suspension. ACI is usually used in intermediate and high-demand patients who have failed arthroscopic debridement or microfracture. The technique can also be used for larger 2 – 10 cm^2 symptomatic lesions. Prior to the surgery, patients must understand and be well prepared to participate in intensive postoperative rehabilitation and should fit the following

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profile: (1) the cartilage damage is focal and not widespread arthritis, (2)presence of pain or swelling that limits everyday activities, (4) a stable knee with no associated ligament damage and (5) normal body mass index (BMI). The postoperative rehabilitation consists of non-weight bearing in addition to range of motion (ROM) exercises with the use of a CPM machine for 6 weeks. Due to two surgical procedures and larger open arthrotomy, pain relief and restoration of function may take as long as 12 to 18 months [109, 110].

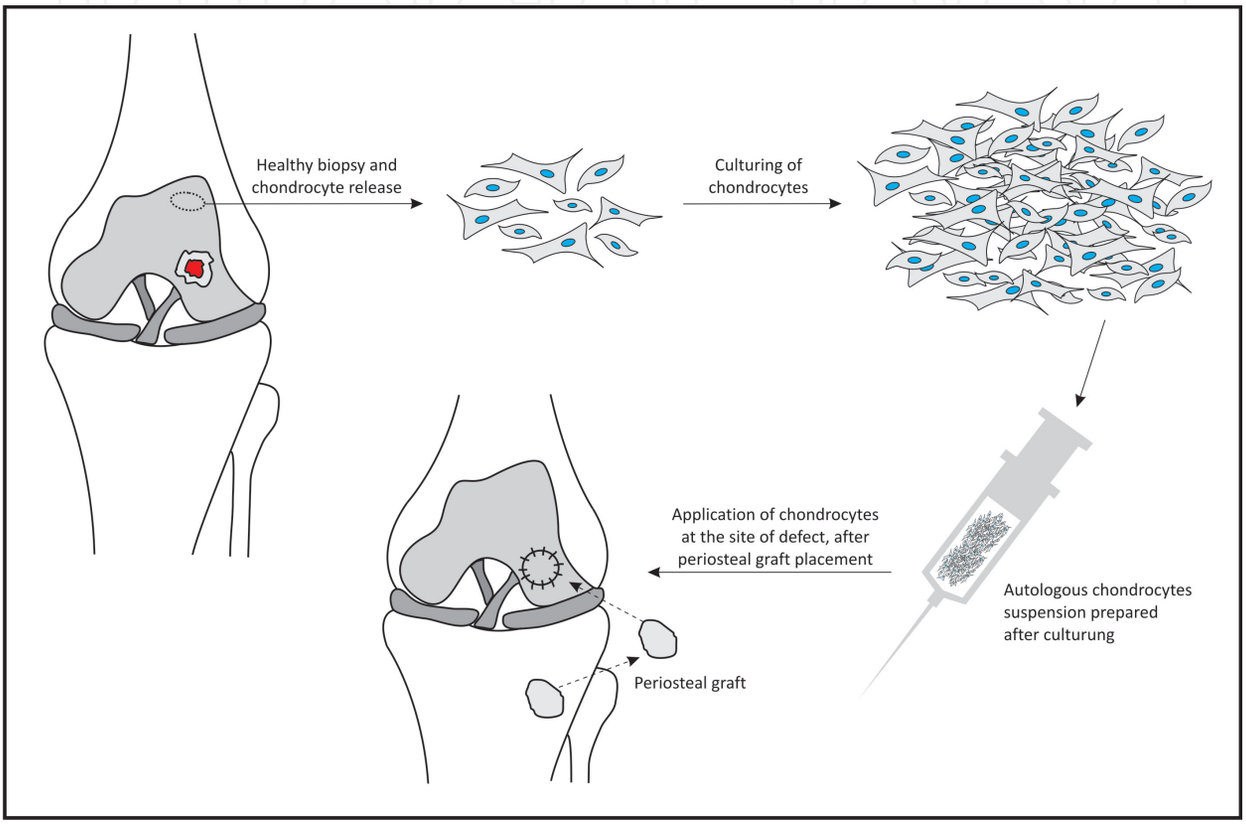

Figure 15. Schematic diagram showing the different stages involved in the process of autologous chondrocyte implantation.

The effectiveness of ACI varies and different levels of success were reported. Recently, ACI has been compared to microfracture technique. Both two and five years follow-up results, after patients were randomized for ACI or microfracture treatment of localized articular lesions of the knee joint, concluded that both methods had acceptable short-term results [111, 112]. There was no significant difference in macroscopic or histological results after two years. Similarly, after five years both methods provided satisfactory results in about 77 % of patients with no significant difference in clinical and radiographic results [112]. Currently, it seems as ACI is as good or a slightly better technique compared to a less invasive, simpler, and cheaper surgical technique in short-term. On the other hand, the significant superiority of ACI over mosaicoplasty for the repair of articular defects in the knee was reported in prospective randomized controlled trails [113, 114].

These results might not be surprising considering the traditional ACI (first generation ACI) encountered several problems. The most common complication with 10-25% incidence is periosteal hypertrophy due to scar tissue formation around the edge of the periosteal patch [115]. In addition, the need of periosteum widens the donor site morbidity and extends the operation time. The periosteum has to be tightly and waterproof sutured to prevent the potential leakage of the cell suspension from the defect. Another frequent disorder is patch delamination due to incomplete bonding of the patch with surrounding tissue. There are several other disadvantages regarding this method: the growth of cartilage tissue is age-dependent (lower potential in the elderly), difficulty to harvest and isolate sufficient numbers of cells from a small amount of tissue removed, fast differentiation of chondrocytes during *In vitro* cultivation in monolayers (loss of phenotype, differentiation in fibroblast-like cells), etc. Reoperation rate as high as 42 % was reported by several authors [116].

4.4. Future prospective for cartilage repair

Some of the problems have been avoided by using the collagen membrane instead of traditional periosteum patch. Anyway, the new technique (ACI-C) has still not solved the problem of watertight sutures and possible leakage. Nevertheless, in randomized control trial the comparison among the two procedures showed a lower re-operative rate in ACI-C, most probably due to the lesser extent of periosteum hypertrophy [117]. The new concept of cartilage tissue preservation was developed using tissue engineering technologies, combining new biomaterials as a scaffold, and applying growth factors, stem cells, and mechanical stimulation [118]. The recent development of so-called second regeneration ACI uses a cartilage-like tissue in a 3-dimensional culture system that is based on the use of biodegradable material which serves as a temporary scaffold for the *In vitro* growth and subsequent implantation into the cartilage defect. It has been shown *In vitro* that the application of 3-D environment promotes hyaline-like cartilage production and allows for mechanical stimulation [119, 120]. Several reports already described a superior role of the MACI (matrix/membrane autologous chondrocyte implantation) and CACI (collagen-covered autologous chondrocyte implantation) compared to the standard ACI procedure [121]. Additionally, the modern concept of tissue engineering uses various types of growth factors which are the endogenous regulators of chondrogenesis and their logical choice of use and relative ease of application have been reported to promote cartilage development [122]. Further studies are attempting to create the ideal scaffold and explore the synergistic effect of concomitant application of growth factors and mechanical loading [120] [123]. Finally, for clinical practice, single stage procedures appear attractive to reduce cost and patient morbidity. These procedures are promising, but there are only a few clinical studies and the results are in the process of publication and will be presented in the following chapter as they represent the most advanced and future therapeutic strategies for cartilage repair.

5. Conclusion

Locomotory system injuries are significant public health problems that contribute to a large burden of disability and suffering worldwide and are the most common injuries encoun-

tered in sports. The management of these injuries in athletes is particularly difficult as they have high demands and expectations. Achieving a fast recovery time and low possibility for reinjury is an ideal goal of each therapeutic team. Neglecting physiological processes in an injured tissue can often lead to inappropriate therapeutical interventions followed by unfunctional regeneration. The importance of keeping in mind the tissue processes at molecular level is therefore crucial and the only way to appropriate therapies.

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