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Tendon and Ligament Tissue Engineering, Healing and Regenerative Medicine

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Abstract

Tendons transmit forces from muscle to bone and provide the joint function and ligaments transmit forces from bone to bone and provide joint stability. Tendon and ligament injuries have high incidence and management of tendon and ligament injuries is technically demanding because the healing response of these soft connective tissues is low. In addition, number of the available options to be considered as tissue replacement for large defects is low and healing of tendon and ligaments is faced to significant limitations. Among the available options, autografts are still gold standard but all the auto- allo and xenografts have their own limitations. Tissue engineering is a newer option but it is still primitive to be applicable extensively, in clinical setting. Tissue engineering could be divided into four categories including scaffolds, healing promotive factors, stem cells and gene therapy. To be able to have a good judgment regarding the management of tendon and ligament injuries, it is crucial to have a basic knowledge of tendon and ligament healing and regeneration. In this review, we discussed various types of tendon and ligament injuries and their incidence, and introduced the available and future options in managing large and massive tendon and ligament injuries. We specifically discussed the tissue engineering and it's advantageous and disadvantageous. To give a better clarification for the readers, we described different phases and cascades of tendon and ligament healing, modeling and remodeling, host-graft interaction after implantation of the graft and various types of prosthetic implants and finally provided some suggestions for the future investigations.

Keywords: Tendon; Ligament; Tissue engineering; Regenerative medicine; Scaffold; Growth factors; Stem cells; Glycosaminoglycans; Healing; Host-graft interaction; Autograft; Rejection; Repair

Introduction

Tendons transmit forces from muscle to bone and provide the joint function [1,2]. Ligaments transmit forces from bone to bone and provide joint stability [3]. The incidence of tendon and ligament injuries is high and several peoples are presented with these injuries to orthopedic surgery [1-5]. Tendon and ligaments are low vascularized tissues and have low healing capacity and capability; therefore management of tendon and ligament injuries is technically demanding [4-12]. Regardless of the acute or chronic nature of the tendon and ligament injuries, surgical reconstruction of the large and massive tissue defects is a more complicated situation [13-21]. There are some conventional available options in managing such large tissue losses including auto- allo- and xenografts [3,13-15]. Autografts are still gold standard while the allo grafts and xenografts have been introduced as alternative options having lower value than the autografts [3,12-14]. All these available options have their own significant limitations [3]. Tissue engineering is a newer option and is divided into four categories of scaffold, healing promotive factors, stem cells and gene therapy [22-32]. Using each or a combination of them have provided the new insights in orthopedic surgery [12,24,30-32]. To design a proper and well approved tissue engineered based graft to be considered as a substitution of traditional grafts, the knowledge about tendon and ligament healing, modeling and remodeling is important [12,24]. In this review we discussed tendon and ligament injuries, have introduced the available and future options in managing large and massive tendon and ligament defects, specifically focused and discussed about the role of tissue engineering in reconstruction of the tendon and ligament defects and finally, described several important events of tendon and ligament healing, modeling and remodeling. Some important and novel concepts about host-graft interaction after surgical implantation of various grafts and prosthetic implants have also been discussed. In addition, some concepts regarding post-operative management and rehabilitation have been highlighted. Moreover we tried to give some suggestions for future regarding the tendon and ligament tissue engineering researches.

Structure of Tendons and Ligaments

Tendons and ligaments have similar structures with different functions [1,3]. Tendon connects muscle to bone and therefore it provides joint movement but ligament connects bone to bone and produces joint stability [1,3,12]. Although these two structures have different duties but both of them have similar characteristics [1,3]. Both are mainly made up of collagen type I molecules and these molecules are arranged as fibrils, fibers, fiber bundles and fascicles [1,3]. Based on the anatomic position of these structures, extra synovial tendons are covered by paratenon and intrasynovial tendons and ligaments are covered by a synovium or synovial sheath (Figure 1) [1-3,12]. In general tendons and ligaments have low cellularity consisting of mature fibroblast or tenoblasts and fibrocytes or tenocytes [1-11]. Thus, more than 90% of the dry matter of tendons and ligaments are collagenous in nature with a little cellularity and other tissue compositions such as glycosaminoglycans and elastic fibers [3,12]. Normally, the proper body of these structures have low vascularity and they are nourished by peripheral vessels mainly presented in the synovial sheath or paratenon [1,12]. Therefore, the metabolic activity of these structures are low as compared to other orthopedic tissues (e.g. cartilage and bone) [1,3,7]. This unique characteristic of tendon and ligament let the tissue to be more tolerable and resistant when the high energy forces are transmitted through the tissue especially during locomotion and physical activates [1-3]. In such conditions the cellular metabolism is changed from aerobic to anaerobic situation and these tissues are more

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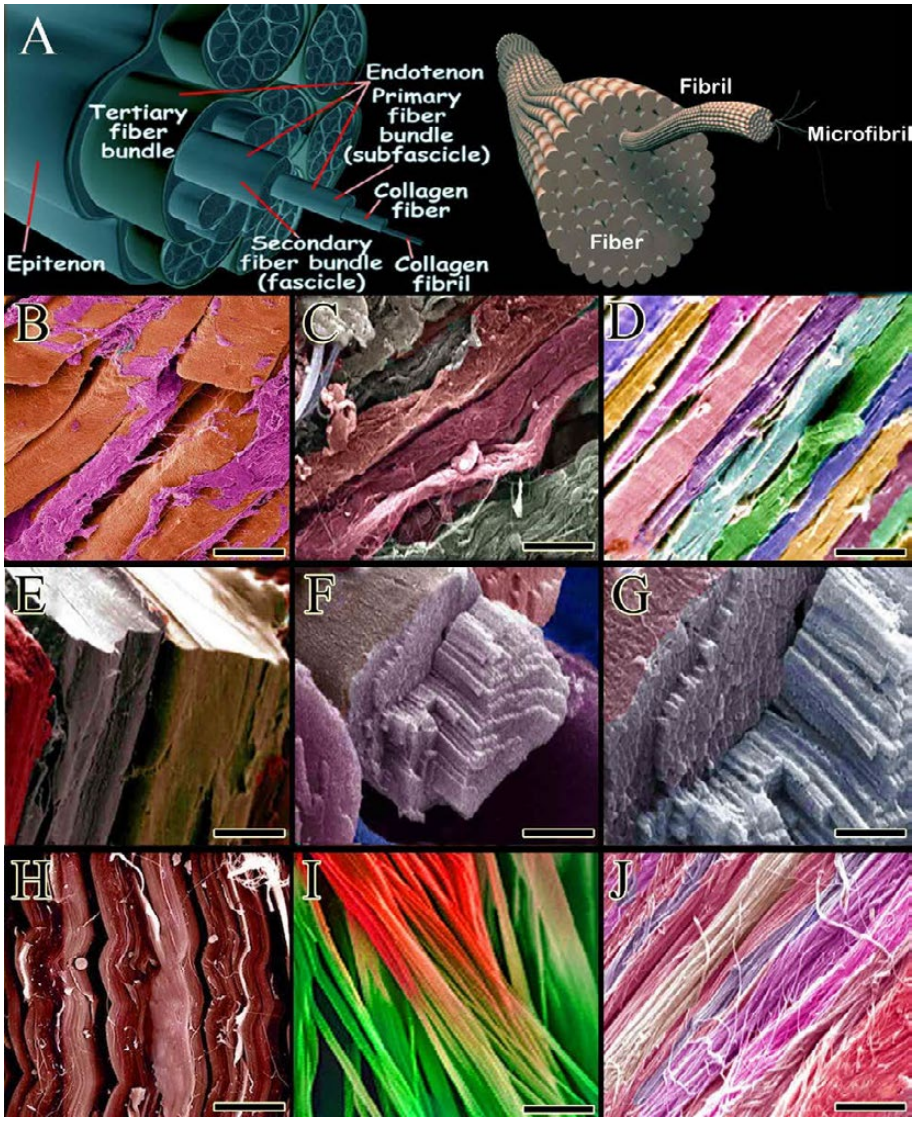

Figure 1: Structure and hierarchical architecture of normal tendon and ligament.
The hierarchical architectures of tendon and ligaments and their tridimensional architectures have significant similarities except that ligaments are denser than tendons and their collagen fibers are twisted. The collagen molecules are polymerized as tropocollagen, micro fibrils, fibrils, fiber bundles and fascicles (A). Endotenon covers the collagen fibers and bundles of collagen fibers internally and epitenon covers the tertiary fiber bundles (the largest bundles of collagen fibers). All these architectures are covered by paratenon in extra-synovial tendons and by tenosynovium or tendon sheath in intra-articular tendons (A). Ligaments (B) are denser than tendons (C, D). (E) is a scanning electron micrograph (SEM) of a normal tendon fascicle. (F) shows a large fiber bundle in which the collagen fibers are densely packed and formed a collagen fiber bundle (F and G). (H) is an ultramicrograph by SEM of the ligament fibers. Normally, these fibers are highly dense and aligned in a unidirectional pattern. (I and G) are the collagen fibrils. The collagen fibrils are aligned in one direction and aggregation of the highly aligned collagen fibrils produces the collagen fibers. B-D and H-J are longitudinal sections while E-G are sagital and cross sections. The ultramicrographs are colored through the structure detection software. Scale bar for B-D: 10 μm, E:100 μm, F:45 μm, G:22 μm, H: 10 μm, I: 700 nm, J: 1.8 μm.

tolerant to anaerobic metabolism compared to other tissues [1]. The low metabolic rate and well-developed anaerobic energy-generation capacity of these structures are essential in carrying loads and maintaining long-standing tensions, and reducing the risk of ischemia and subsequent necrosis [1]. Although this is an excellent tissue characteristic for the normal state of these structures but this could be a negative point in the tendon or ligament injuries and healing [1-3].

Tendon and Ligament Injuries

Tendon and ligament injuries have high incidence in orthopedic surgery and sports medicine [12-21,33,34]. Tendon and ligament injuries are vary in nature and could mainly be divided into acute and chronic injuries [1,12]. In some of the acute tendon or ligament injuries, a simple transection is occurred as a result of sharp traumas or during routine orthopedic surgeries (e.g. bone fixation using plating techniques; tissue dissection, etc.) [4-8]. In addition, these types of injuries have been reported to occur as a result of vehicular trauma [9-11]. In orthopedic surgeries, in order to provide the maximum exposure to bones and joints, it has been suggested to keep the tendons and ligaments free of sharp dissection and it is advised to dissect these structures from their bony insertion [3,12]. This situation is a common condition when exposing joints [3]. Despite of the simple nature of this model of injury, current literature suggests a poor healing response is occurred [7]. Basically, it is a rule to reapposite the transected tendon

edges and suture them [4,5]. In the Achilles tendon, simple end-to-end repair is most commonly performed via the Bunnel, Kessler, and Krackow techniques [35]. All these suturing techniques are acceptable for the repair of the Achilles tendon; however, the Krackow method has been shown to be superior in biomechanical and cadaveric studies and has the advantage of allowing 4 threads (and 2 knots) across the rupture site if desired [36].

However, such model of direct suturing is not suggested for the ligaments. It has been stated that direct suturing has low value in torn ligaments because the torn ligaments would not heal in the intra articular space and the inflammatory environment of the joint is degenerative in such condition which do not let an acceptable healing to be processed [3]. Ligament replacement is the best option [3]. The second form of acute tendon or ligament injuries are unsharp or blunt ruptures [12]. This type commonly occurs when high energy forces greater than the ultimate strength of the tissue passes through it [12]. Basically, these high energy forces are produced as a result of high physical activities (e.g. athletics) [3,12]. These sharp tissue ruptures are called strain injuries when a tendon is ruptured or called sprain injuries when a ligament is ruptured [1-3]. Strain and sprain injuries are mainly divided into three degrees [12]. Few collagen fibrils and fibers are microscopically ruptured in degree one but the tissue injury is not obvious at gross morphology [1]. In the second degree, a considerable number of collagen fibrils, fibers and bundles of collagen fibers are ruptured and a partial gap between the ruptured fibers is grossly visible [12,33]. In this condition, the continuity of the tissue in not completely lost [33]. Finally, in the third degree, total collagen fibers and bundles are ruptured and a considerable gap occurs between the edges [25-29]. Treatment of grade one is non-surgical and is limited to supportive strategies; however there is no well accepted method for managing grade two injuries (surgical vs. non-surgical) and in grade three (total rupture), in most instances the ruptured tissue should be surgically repaired [12,29]. Other tendon injuries commonly happen due to traumas, burns, gangrenous and infective ulcers, tumors and several other predisposing factors and mainly result in tissue necrosis [29]. In such conditions it is often necessary to resect the remaining tendon and this results in gap formation [12,29]. The gap should be surgically reconstructed and if it is neglected then joint stiffness occurs which impairs the functionality of the injured limb [26]. Despite of recent advances in orthopedic techniques, however reconstruction of such large and massive tissue defects is technically demanding and variation in surgical techniques in repairing such tissue defects are remarkable and mostly it is hard for surgeons to choose the method of choice [12,26,29]. Many different surgical techniques exist, but only a few of them have been validated in a strict scientific manner [13-21]. The V-Y technique, local tissue augmentation, turn-down flaps, tissue transfer, free tissue transfer, and use of synthetic materials are some of the examples [13-15,29]. Each has its own significant limitations [14-19]. Here we described some surgical techniques useful in Achilles tendon reconstruction.

End-to-end repair with augmentation can be performed with additional autografts, allografts, and synthetic grafts. Graft options have been discussed separately in the following sections of this review. The most common and easiest autograft to harvest is with the plantaris tendon, which is usually intact after a rupture and easily available through the same surgical wound. The tendon is cut from its insertion on the calcaneus and stretched or "fanned out" and can be placed over the rupture site with absorbable sutures [37-39].

Fascial turn down flaps of the proximal gastrocnemius fascia have also been advocated. The most common being the single strip or Silfverskio¨ ld procedure and the double strip or Lindholm procedure, and these fascial slips are made approximately 3 cm proximal to the rupture site and rotated 180º and flapped down to cover and reinforce the rupture site. It should be noted that doing a turndown flap requires a longer proximal skin incision with more dissection that increases the potential for wound healing issues and sural nerve injury [40].

Minimal incisional and percutaneous techniques were introduced and advocated to decrease potential wound complications, scar adhesions, and sural nerve injuries [41-43]. Ma and Griffith first described a percutaneous technique using multiple stab incision of 18 acute Achilles tendon ruptures to minimize postoperative complications and reported no complications of sural nerve injury [41]. It should be noted that percutaneous repairs are not completely benign and every surgeon should appreciate this and use caution when placing a proximal and especially proximal lateral incision even with this technique. Martinelli described percutaneous repair on 50 acutely ruptured Achilles tendon [42]. In this study, 30 patients practiced amateur or professional sports and were able to return to preinjury sporting levels after 120 to 150 days. Favorable arguments for mini-open technique are allowing for early postoperative ROM and rehabilitation to assist proper tendon healing with the reduction of scar adhesions and allowing athletes an earlier return to sporting activities.

Among all chronic tendon injuries, tendinopathy has the highest incidence and importance [1]. Excessive loading on tendons during vigorous physical training is regarded as the main pathological stimulus for degeneration, and there may be a greater risk of excessive loading inducing tendinopathy in the presence of intrinsic risk factors [12]. Tendons respond to repetitive overload beyond the physiological threshold with either inflammation of their sheath or degeneration of their body, or both [1,2]. Different stresses induce different responses [19]. Unless fatigue damage is actively repaired, tendons will weaken and eventually rupture. The repair mechanism is probably mediated by resident tenocytes, which maintain a fine balance between extracellular matrix network production and degradation [4]. Tendon damage may even occur from stresses within the physiological limits, as frequent cumulative microtrauma may not allow enough time for repair [12]. Microtrauma can also result from nonuniform stress within tendons, producing abnormal load concentrations and frictional forces between the fibrils and causing localized fiber damage [1]. The etiology and importance of tedinopathy has been extensively discussed [1].

Epidemiologic Aspects of Tendon and Ligament Injuries

Of the 33 million musculoskeletal injuries reported in the United States per year, roughly 50% involve injuries to the soft tissue including tendon and ligament [1,13,44,45,47-50]. As larger portions of the general population participate in physical and recreational activities every year, the frequency of tendon and ligament injuries is likely to increase as well, resulting in increasing health care costs and patient morbidity [1-5,46,48]. Twenty-five percent of all athletic injuries, irrespective of the specific sport or level of play, involve the foot and ankle [14,15,44]. Here, some epidemiologic aspects of tendon and ligament injuries have been shortly discussed.

Achilles tendon ruptures were apparently rare before the year 1950, but the incidence has recently increased in developed countries [15,51]. A bimodal age distribution (peak in the fourth decade of life followed by a second, but lower, peak in the sixth to eighth decade of life) among patients with a rupture has been noted in most of the studies, and this probably represents two different etiologies of Achilles tendon rupture [16-19,51]. Especially in the younger age group, the majority of Achilles tendon ruptures are related to sports that require sudden acceleration

and jumping [19]. It has been shown that 755 of nearly 4000 ruptures of the Achilles tendon were related to sports activities [21,44]. The incidence of Achilles tendon rupture in men is about 1.7 to 7 times greater than in women [15,16]. The first peak in incidence of Achilles tendon rupture, is between 30 and 40 years of age, lower than the age in patients with other spontaneous tendon ruptures [1,15,44,51]. In some studies, the second peak has been suggested to be between 50 and 60 years and in some others between 70 and 80 years, closer to the mean age of patients with other types of tendon ruptures [19,20,45,47].

The distribution of Achilles tendon rupture by different sports varies considerably from country to country [15,44]. For example, in northern and middle Europe, soccer, tennis, track and field, indoor ball games and downhill skiing and in North America, football, basketball, baseball, tennis and downhill skiing dominate the statistics [15-17,44,51,52]. However, during the last decade, it has been suggested that ball games covered about 90% of all sports related Achilles tendon ruptures and the incidence of badminton induced ruptures is increasing yearly, so that this sport is now placed in the first cause of Achilles tendon rupture in Finland and Sweden [15-17,51,52]. In some other countries, downhill skiing has been shown to be the most common cause of Achilles tendon rupture. Although people's general lifestyle has become more sedentary and physically less demanding during recent decades, increasing leisure time, and especially increased recreational and competitive sport activities, has resulted in a greater incidence of acute and overuse sports injuries [53,54].

Chronic problems caused by overuse of tendons probably account for 30% of all running-related injuries, and as an example, the prevalence of elbow tendinopathy in tennis players can be as high as 40% [1,50]. The occurrence of Achilles tendon overuse injuries is the highest in middle- and long distance running, orienteering, track and field, tennis and other ball games [53,55]. An annual incidence of Achilles tendon overuse injuries in top level runners has been shown to be of 7 to 9% [55]. The most common clinical diagnosis of Achilles overuse injuries is tendinopathy of the main body of the tendon (55 to 65%), followed by insertional problems such as retrocalcaneal bursitis and insertion tendinopathy (20 to 25%) [55]. The etiology of Achilles tendon overuse injuries is multifactorial [1]. Training errors have been reported in 60 to 70% of the running injuries [55]. The most common training errors associated with Achilles tendinopathy as for many other overuse injuries are a rapid increase in mileage, increased intensity of training, and running on sloping and slippery roads [53,56]. The symptoms of Achilles tendon overuse injuries are usually initially unilateral however it has been suggested that the patients with unilateral tendinopathy seem to have a relatively high risk (reported to be about 41%) to get the symptoms of overuse (exertional pain with or without swelling and stiffness) to the initially uninvolved contralateral Achilles tendon, too [52].

About one-third of sport injuries that are treated at outpatient sports clinics involve the knees [57]. Patellar tendinopathy has high incidence in knee disorders that are yearly presented in the clinics [58]. It has been shown that the highest incidence is in soccer (21%), long distance running (13%), volleyball (12%), orienteering (8%) and ice hockey (7%) [58]. Men are affected about 4 to 6 times more than women [57]. Overuse injuries of the patellar tendon are most common in athletes involved in some types of repetitive activities, such as jumping (volleyball, basketball, high jump, and triple jump), kicking (soccer, football), quick stops and starts (tennis, squash, badminton), running (sprinters, endurance runners), weightlifting and powerlifting, and bicycling [58]. It has been shown that increased laxity of the knee joint correlated with patellar tendinopathy [58]. The pathologic changes are most often located at the insertional areas, where micro- or macroruptures and tissue degeneration are often found. In volleyball, landing from jump (eccentric loading) and repeated direct striking of the knees on the floor may play a role in the etiology of the patellar tendinopathy [57,58].

A rotator cuff tendon injury involves any type of irritation or damage to the rotator cuff tendons [59]. This can be caused from traumatic injuries (falling on an outreached arm), lifting, or repetitive arm activities done overhead, such as throwing a baseball or placing an item on a shelf [60]. These injuries can be as simple as a strain or tendonitis, and as severe as partial or complete tear of the tendon [59]. The incidence of rotator cuff tendon damage increases with age and is most frequently due to degeneration of the tendon, rather than injury from sports or trauma [59-61]. Though found to be more common in older populations, rotator cuff injuries do not discriminate and occur in males and females of all ages [61,62]. Due to the aging process, the rotator cuff tendon tissue loses its elasticity, become more susceptible to injury, and is more frequently damaged while performing everyday activities [63]. In younger patients, these injuries are typically due to unusually high demand of the shoulders or traumatic injuries [59]. In addition to primary causes of injuries, the risks of developing weakness or injury to the rotator cuff include: being an athlete (using repetitive motions, such as baseball pitchers, swimmers, archers and tennis players), working in the construction trades (carpenters and painters), poor posture, and weak shoulder muscles [60-63].

Flexor tendon injuries are common events as the tendons lie close to the skin and so are usually the result of either lacerations such as those from knives or glass, from crush injuries and occasionally they can rupture from where they are joined to bone during contact sports such as football, rugby and wrestling [2,64,65]. Flexor tendon injuries are challenging problems for orthopedic surgeons due to three main reasons [2,4,64]. Firstly, flexor tendon injury in hand is a clinical problem because it cannot heal without surgical treatment, as the two ends need to be surgically brought together for the healing to occur unlike other tendons including the Achilles tendon where it could be placed into plantar flexion to heal [8-11,64]. Secondly postoperative management needs to be carefully planned as mobilization has shown to be essential to prevent adhesions and improve gliding but this can risk rupture. Lastly due to the unique anatomy of the tendons running through flexor tendon sheaths to function, surgeons need to plan preventing increasing the bulkiness of the tendon through its sheath, which is not always possible from scarring as this affects the functional outcome of the tendon [2,5-7,64]. Unfortunately there is no well-known incidence of flexor tendon injury but to the authors experience it seems to be high both in humans and animals.

Among all the ligament injuries which are yearly presented in the orthopedic practice, ACL has been shown to have the highest incidence [66]. Ligament injuries are most often a result of low-velocity, noncontact, deceleration injuries and contact injuries with a rotational component [67]. Contact sports also may produce injury to the anterior cruciate ligament secondary to twisting, valgus stress, or hyperextension all directly related to contact or collision. When matched for activities, a greater prevalence for ACL injury is found in females compared with males. In one meta-analysis study by Prodromos et al. [34], it has been shown that female subjects have a roughly 3 times greater incidence of ACL tears in soccer and basketball versus male subjects. They showed that recreational Alpine skiers had the highest incidences of ACL tear, whereas expert Alpine skiers had the lowest incidences. Volleyball may in fact be a low-risk sport rather than a high-risk sport. Alpine skiers and lacrosse players had no gender difference for ACL tear rate. Year-

round female athletes who play soccer and basketball have an ACL tear rate of approximately 5% [34].

Approximately 50% of patients with ACL injuries have meniscal tears too. In acute ACL injuries, the lateral meniscus is more commonly torn; in chronic ACL tears, the medial meniscus is more commonly torn [3]. The incidence of ACL tears has been estimated as 1 case in 3,500 people, resulting in 95,000 new ACL ruptures per year [66]. The importance of the ACL has been emphasized in athletes who require stability in running, cutting, and kicking. The ACL-deficient knee has also been linked to an increased rate of degenerative changes and meniscal injuries. For these reasons, approximately 60,000-75,000 ACL reconstructions are performed annually in the United States [66]. For restoration of activity and stability, the expected long-term success rate of ACL reconstruction is between 75-95%. The current failure rate is 8%, which may be attributed to recurrent instability, graft failure, or arthrofibrosis [3,34].

Managing Mild Tendon Injuries with Least Tissue Defect

In order to manage mild tendon injuries, several factors have a role on the final decision [44]. Physical therapy, rest, and gradual return to the activity in which tendinosis was experienced is a common therapy [1,46,47]. There is evidence to suggest that tendinosis is not an inflammatory disorder; anti-inflammatory drugs are not an effective treatment, and inflammation is not the cause of this type of tendon dysfunction [48]. There is a variety of treatment options, but more research is necessary to determine their effectiveness [48,49]. Initial recovery is usually within 2 to 3 months, and full recovery usually within 3 to 6 months. About 80% of the patients will fully recover within 12 months. If the conservative therapy doesn't work, then surgery can be an option [50]. This surgery consists of the excision of abnormal tissue and reconstruction of the defect area [13-20]. Time required to recover from surgery is about 4 to 6 months [15].

Controversy regarding operative versus non-operative treatment exist in the treatment of tendon ruptures that focuses on re-rupture rates and wound complications related to surgery [15,35-38,54]. Re-rupture rate of 3.5% in the operatively treated patients and 12.6% in the non-operative patients has been reported previously [38]. Complications such as adhesions, altered sensation, and wound infection, however, were more common in operatively treated patients with 34.1% in the operative patients versus 2.1% in the non-operative patients [39]. Due to the similar percentage of re-rupture between most studies advocating surgery and their promising results with non-operative management, the necessity for surgical repair of Achilles tendons, with all of its risks and high cost is questionable [38]. Therefore, for mild tendon ruptures with the least tissue loss, conservative or non-surgical or non-invasive treatment may be a first line approach [15-17,54]. Patients with pain, whose function is reasonably maintained, are suitable candidates for non-operative management [38]. A conservative physical therapy program begins with preliminary rest and restriction from engaging in the event which gave rise to the symptoms [44,48,50]. Oral medications that provide pain relief such as anti-inflammatory agents, topical pain relievers such as cold packs and, if warranted, local anesthetic injection may be used [50,54]. A sling may be offered for short-term comfort, with the understanding that undesirable joint stiffness can develop with prolonged immobilization. Early physical therapy may afford pain relief and help to maintain motion. As pain decreases, strength deficiencies and biomechanical errors can be corrected [54,55]. A gentle, passive range-of-motion program should be started to normalize the stiffness and maintain range of motion during this resting period [55,58]. Exercises should be a part of this program [54]. After a full, painless range of motion has been achieved, the patient may advance to a gentle strengthening program [51].

There are several instances in which non-operative treatment would not be suggested [15,34,52,54]. The first is the 20 to 30-year-old active patients with an acute tear and severe functional deficit from a specific event [54,59]. The second is the 30 to 50-year-old patients with acute tendon tear secondary to a specific event [61,63]. The third instance is the highly competitive athlete who is primarily involved in overhead or throwing sports [51,54]. These patients need to be treated operatively because tendon repair is necessary for restoration of the normal strength required to return these athletes to the same competitive preoperative level of function [51]. Patients who do not respond or are unsatisfied with conservative treatment should seek an opinion concerning surgery [54]. Patients associated with significant tendon defect should be surgically treated and the defect area should be surgically reconstructed by grafts [29].

Managing Large Tendon and Ligament Injuries

Patients associated with significant tendon defect should be surgically treated and the defect area should be surgically reconstructed by grafts [15-18]. Also most of the torn ligaments especially the intrasynovial ones such as ACL should be surgically replaced by a graft and therefore no medical or conservative treatment option is warranted for these patients [3,23,34,35].

Graft Options In Managing Large and Massive Tendon and Ligament Injuries

Using autografts is currently the only gold standard method for managing large tendon and ligament deficits [26,29]. However, reconstruction of such large defects requires massive tissue harvesting from the donor site and therefore the donor site morbidity and pain is a considerable challenge to autografts [3,12-14]. In addition there may not be enough autografts in the patient's body at the time of reconstruction and cosmetically this method is not pleasant [3,13]. Another limitation of this treatment strategy is that it is commonly necessary to make a second surgery in the patient which increases the surgical time and cost [15]. In contrast to autografts are allo- and xenografts [13,16]. Allografts have lower immunogenicity than xenografts but both of them have this chance to be acutely or chronically rejected by the host [12]. Thus rejection is one of their limitations [68-71]. In addition, it has been stated that using allo- or xenografts increases the chance of disease transmission of several hazardous and dangerous viruses and prions such as human immune deficiency virus, hepatitis type B and more importantly type C and bovine spongiform encephalitis [3,12,26,29]. Disease transmission in xenografts is a more serious concern than allografts because there are well known human diseases that could be detected in the allografts using the standard screening technologies but in xenografts there are several unknown zoonotic diseases that are more hazardous for the humane body than those of the well-known humane diseases [3,12,29]. In addition to these disadvantageous there are also several ethical concerns regarding usage of allo- and xenografts in the human body [13-16].

Comparing auto- to allo- and xenografts options, autografts have better incorporative properties than the other grafts because basically the autografts are viable tissues and their cells can collaborate at different stages of healing [3,71-73]. In addition the resorption rate of autografts are lower than other grafts; therefore the healing in autografts would be faster [3,72]. In contrast to autografts, the most important positive and beneficial issue regarding allo- and xenografts

is their availability [13-15]. However, to reduce the immunogenicity of the allo- and xenografts, several processes have been made (e.g. sterilization, cell rinsing, freeze drying, etc.); therefore these types of grafts are not viable in nature because their cellular structures are commonly destroyed during sterilization and other important tissue processing [72-85]. Therefore, it is a fact that the allo- and xenografts have significantly lower incorporative properties with the healing tissue and this is another major limitation which results in rapid absorption of the graft during tendon or ligament healing [69,72].

Tissue Engineering

Tissue engineering is another option which has been introduced in the last decade and currently it is the final option in managing large and massive tendon and ligament injuries [22-32]. Tissue engineering is defined as "a process that affects the structure and architecture of any viable and nonviable tissue with the aim to increase the effectiveness of the construct in biologic environments" [12]. Therefore, all of the non-fresh grafts which are processed for acellularization belong to the tissue engineering category [68-72]. In fact, acellularization is the basic tissue engineering technology as described for the allo- and xenografts [68,70,72]. This method of tissue engineering has been used for many years to decrease the antigenicity of the viable grafts [68-72]. Newer approaches have been developed by many and newer tissue engineered products are introduced in the recent years [73-85]. Basically, tissue engineering could be divided into four major categories including tissue scaffolds, healing promotive factors, stem cells and gene therapy [12]. The fourth category "Gene Therapy" has been recently included in tissue engineering [12,24].

Scaffolds

Scaffolds are the most important part of tissue engineering because they have many applications and without them application of promotive factors and stem cells have low clinical value because their long term availability without attachment to the scaffolds is questionable [12,22-32]. Tissue scaffolds have several duties but their major role is to provide a suitable environment for cell attachment, migration, proliferation and matrix remodeling and regeneration [12]. In addition, tissue scaffolds have been used in drug delivery, vehicle for healing promotive factors and stem cells and also as tissue filler [24-32]. Based on their stiffness, scaffolds could be divided into three major categories including hard, soft and hybrid scaffolds [12,24]. Based on the source, tissue scaffolds could be divided into three major categories including biologic, synthetic and hybrid scaffolds [22-24]. The acellularized cadaveric tissues or grafts are the most well-known biologic scaffolds [24,68-73,75-81]. However, the newer ones are those constructed by the biologic based molecules such as collagen, elastin, chitosan, demineralized bone matrix, fibrin, gelatin, hyaluronic acid, chondroitin sulphate, silk, etc [12,25-28]. In contrast to biologic scaffolds, the synthetic scaffolds are produced by polymerization of the synthetic materials [12,28,29]. The major category of synthetic based scaffolds are the oil based polymers such as those constructed by polypropylene, polycaprolacton, polydioxanone, polygalactin 910, nylon, etc [12,29]. Other synthethic based materials used to construct synthetic tissue scaffolds are carbon fiber, hydroxyapatite, several forms of calcium phosphate (e.g. mono, bi, tri and octa calcium phosphate), ceramics, etc [12].

Both the biologic and synthetic based scaffolds could be divided into two major categories based on their absorbability [12,24,26]. As a general rule, materials such as collagen, gelatin, elastin, fibrin, hyaluronic acid, chondroitin sulphate, polydioxanone, polygalactin 910, polyglycolic acid, and several forms of hydroxyapatite and calcium phosphates are absorbable materials and the other materials such as silk and nylon are none absorbable materials in the host environment [12,24]. Among the above mentioned absorbable materials the synthetic polymers such as polydioxanone have higher biocompatibility than collagen, elastin, fibrin, etc [86]. This is because the polymer based biodegradable materials are absorbed through a hydrolysis mechanism [12,24,86]. In this mechanism the polymers are degraded to Carbone dioxide and water and therefore this polymer based scaffolds have least tissue reaction after their surgical implantation in the host [12]. In contrast to synthetic polymer based scaffolds, the biologic based polymers such as collagen and elastin are absorbed through a different mechanism which is named, phagocytosis [87,88]. In fact, the exogenous biologic based absorbable scaffolds such as those constructed by collagen Type I, accelerate the inflammation because their surface antigens chemotactically attract the neutrophils and macrophages and this lead to neutrophil and macrophage accumulation in the tissue scaffold [76,77,87]. Therefore, the absorption rate of the biologic based scaffolds are much faster than those of the synthetic based biodegradable scaffolds [28,29]. Similar behavior has been suggested for the none absorbable materials in which the silk has been shown to be more immunogenic than nylon after surgical implantation in the host [12,24]. Despite these explanations, it should be highlighted that the biologic based biodegradable scaffolds such as those constructed by collagen have an acceptable biocompatibility but also they have much better healing incorporative capacity and biodegradability [12,24,29,69,72]. One of the most important goals in tendon and ligament tissue engineering is that the scaffolds should simulate native tendon characteristics in short term but should be gradually degraded and replaced by the new tissue [29]. This newly developed tissue should be comparable to the normal tissue in the least possible time in order to be able to carry out its duty [12].

Synthetic scaffolds have another superiority over the biologic scaffolds and the synthetic based scaffolds have higher biomechanical characteristics than the biologic based scaffolds; therefore application of the synthetic scaffolds has superior outcome at short term while the biologic based scaffolds have better outcome at long term [25-29]. This is a very important concept in reconstructing large and massive tissue defects with tissue engineered based scaffolds [12]. After implantation of a tissue engineered graft, the graft should have an optimum biomechanical property in order to be able to resist against functional weight bearing forces and physical activities of the patient [12,24,29]. The higher biomechanical properties of a scaffold, the lower incidence of dehiscence and gap formation at short term [27,29]. However, as mentioned, those scaffolds with the higher biomechanical properties have lower degradation rate and healing incorporative properties which leads to dehiscence and rupture of the healing tissue at long term [12,24]. This is because the biomechanical properties of the biodegradable scaffolds decreases by time and if a newly developed tissue would not be able to completely substitute the graft, then the rupture could occur [27]. To overcome these limitations regarding the outcome of biologic materials at short term and long lasting synthetic materials at long term, scientists have invented the hybrid scaffolds in which they combined the synthetic materials with biologic materials to increase the biodegradability, biocompatibility, biomechanical properties and healing incorporative characteristics of the implant in order to overcome the above mentioned limitations [22,23,29,75].

In addition, technically it is possible to increase the biomechanical properties of the natural based scaffolds such as collagen [28,75,80,82].

Cross linking of the scaffold fibers is one of these technologies (Figure 2) [80,82]. It is possible to crosslink the scaffold fibers, using various techniques including dehydrothermal, chemical, ultraviolet, etc. [26-29]. Among these well-known technologies, chemical crosslinking has been suggested as the most effective method but crosslinking in chemical solutions such as glutaraldehyde and formalin has been reported to be hazardous for the host because the remaining solvent in the scaffold may be preserved even after several washings [26-29]. Dehydrothermal crosslinking has a low value because it has least effectiveness on the biomechanical properties of the graft but it can decrease the biodegradability of the implant so that by dehydrothermal crosslinking it is possible to increase the persistence time of the implant in the host [27]. The newer approach in cross linking is UV method which is a safe and effective method in both sterilization and crosslinking of the implant with no deleterious effects in the host [25-28,82]. As a general rule it is important to make some degrees of cross linking in the scaffolds in order to increase the persistence time of implant in the host with the aiming to increase the effectiveness of the implant at various stages of healing [12,24,27]. Without cross linking the biologic scaffolds are soluble in water and therefore they are acutely absorbed by the host immune response [28,29].

By combining the biologic based materials with the synthetic materials it is therefore possible to decrease the crosslinking degree but also increase the biomechanical properties of the scaffold [12,29]. Well comprehensive detailed studies have shown that it is better to construct the main part of the scaffold with biologic based materials such as collagen and cover it with a synthetic based scaffold such as polydioxanone [24-29]. This strategy has been shown to be as effective as the natural autografts *in vivo* [24,29]. For example, to simulate tendon proper, a novel tridimensional collagen scaffold has been made and covered with polydioxanone sheath to simulate paratenon [28,29]. It has been suggested that the new tendon have formed through the polydioxanone sheath and at long term no part of collagen implant existed in the defect area and has totally been replaced by the new tendon that had optimum biomechanical properties at long term [28,29]. It has been shown that polydioxanone sheath can provide the initial biomechanical characteristics required for the short term weight bearing which is substituted by the long term biomechanical performance due to the newly regenerated tendon [29]. Another possible strategy is to reduce the weight bearing forces from the tissue scaffold and transfer it on a suture material [27]. This has been shown to be effective in short term, *in vivo* [27]. It is also possible to reduce the weight bearing by casting the injured limb [4-11]. However, the first and second strategies are more advantageous than the latter one because casting the injured limb increases the development of peritendinous adhesion, muscle fibrosis and atrophy [2]. As a new strategy it is possible to chemically combine the synthetic and biologic based materials and produce a new scaffold with hybrid polymeric fibers [12,24]. This is a new area of research and its effectiveness on the biomechanical properties and the healing incorporative capacity of the graft is not clear as yet [12].

In designing a tissue engineered scaffold for tendon and ligaments it is important to consider the above important issues [12,22,23,26]. As suggested, the implant should have some degree of crosslinking to have optimum biomechanical properties at short term but also it should be cytocompatible *in vitro*, biocompatible, biodegradable and healing incorporative *in vivo* [12,24,26-29]. These are general guidelines [12]. In addition to the above features, the architecture of the scaffold is also important (Figure 2) [1,25]. Tendon and ligaments are highly aligned tissues (Figure 1) and the collagen fibrils and fibers are unidirectionally arranged and are densely packed [4-11]. In addition, these tissues consist of nano and micro collagen fibrils and fibers [12]. In designing a scaffold for tendon and ligament replacement it is important to produce an aligned scaffold and the scaffold should have both micro and nano scaled fibers in its architecture (Figure 2) [12,89].

As another issue, tendon and ligaments are tridimensional structures but their sheath is a bidimensional structure [25-29]. This should be considered that the healing cells have better migration, proliferation and production properties in tridimensional environments [29]. As another factor, the scaffolds should have porosity in their internal architecture and regarding current literature the pore size could be lower than those used for bone and cartilage tissue engineering [12,24]. Therefore the pore size could vary but should consists of pore diameters between 10 to 100 micrometer [12,24,25-29]. In addition the internal pores should

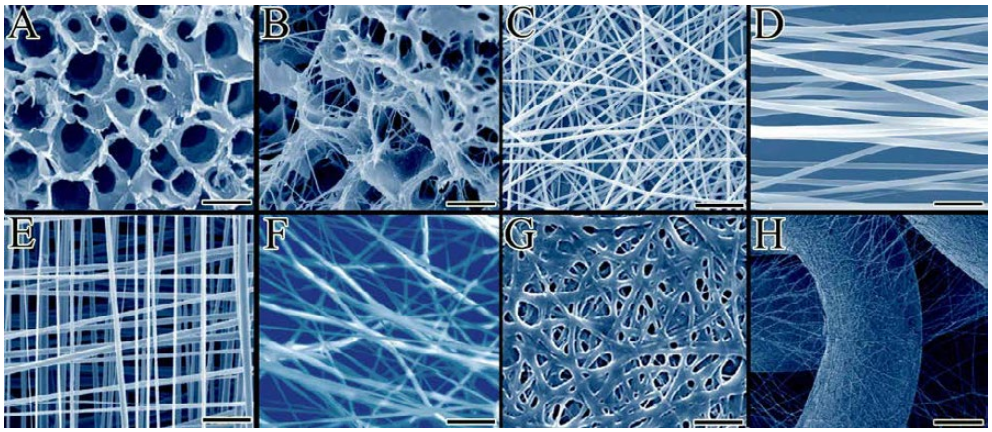

Figure 2: Ultramicrographs by SEM of different tissue engineered based scaffolds.
(A and B) are porous scaffolds. These types of scaffolds are porous in nature and their porosity is high. The pore size is usually between 10 to 100 µm but it may be larger. No fiber structure is visible in these scaffolds and the molecules are not arranged as fibers. The density of the pores is more than 50% in these types of scaffolds. (C to F) are fibrous or fiber based scaffolds. (C) is an amorphous scaffold which has some porosity between its fibers while (D) is a highly aligned scaffold which has lower porosity than (C). (E) is a unique highly aligned scaffold but the fibers have been polymerized and aligned in two different directions so that some of the fibers are perpendicular to others. This unique configuration of the fibers has produced large pores between the fibers. (F) is a moderately aligned scaffold in which the collagen fibers are mostly aligned but an acceptable porosity exists between the fibers. (G) is a fibrous scaffold which have been cross-linked so that the fibers have been fused together but some porosity is still preserved in the scaffold. (H) is a hybrid scaffold in which both the micro and nano structured fibers are present in the scaffold. Scale bar for A: 1 µm, B: 500 nm, C to F: 1 µm, G: 2 µm, H: 2.4 µm.

be connected with the external pores at only two sides of the scaffold [12]. These are those sides that are laid between the tendon or ligament edges after graft implantation *in vivo* [89]. The surface of other sides of the scaffold should be none porous because porosity at other sides could accelerate peri-tissue adhesion formation [12,89].

Careful attention should be paid on the sterility and endotoxin content of the scaffolds [84]. Several hazardous solvents and microorganisms could contaminate the implant during production and therefore quality control tests regarding the sterility and endotoxin content should be performed before surgical implantation [12,27]. As a general rule the scaffolds could be sterilized with ethanol 70% for 1 hr [29]. However ethanol has some effect on tissue architecture and it may not be sufficient to kill all the microorganisms such as bacterial spores [12]. Newer technologies have been introduced and among them UV and gamma irradiation have been found much effective in eliminating all the contaminations [12,24,83,84].

Healing Promotive Factors

''Healing Promotive Factors" is another category of tissue engineering [12,30,31]. These are basically divided into four categories including glycosaminoglycans, growth factors, pro-inflammatory mediators and healing agents [12,30,31]. Glycosaminoglycans are present in the tissue matrix of all the connective tissues [4,9,31,90]. Hyaluronic acid, chondroitin sulphate, dermatan sulphate, keratin sulphate and glucosamine are some examples of this category [1]. Glycosaminoglycans have several important characteristics in tissue healing and remodeling [4,9]. For example, hyaluronic acid has been shown to modulate the inflammation at short term but also has a role in collagen fibril aggregation and development in later stages of tendon or ligament healing [4,9]. It can reduce cell proliferation over the tendon and ligament surface thus has a role in decreasing peritendinous adhesion and it also increase cell migration and proliferation in the internal parts of the injured tendon and ligaments and therefore it can increase the healing quality of the injured tissues [9,31]. Poly-sulphated glycosaminoglycans such as chondroitin sulphate has been shown to increase the collagen fibrils' diameter and aggregation and have a role in matrix remodeling at later stages of healing [4]. Although the major role of glycosaminoglycans is to increase the healing quality of the regenerated tissue but they have been used as architectural polymers in combination with other materials such as collagen and elastin [12,24,31]. Therefore, this category has an important role in tissue engineering [31].

During tissue repair growth factors play an important role in regulating several events [30]. To date, several growth factors have been characterized and introduced [91-99]. The main important well-known growth factors are basic and acidic Fibroblast Growth Factor (FGF), Vasculoendothelial Growth Factor (VEGF), Transforming Growth Factor beta (TGF-β), Platelet Derived Growth Factor (PDGF), bone morphogenic protein (BMP), etc. [3,5,6,10,30]. The role of basic fibroblast growth factor has been well shown previously [5,6,10]. FGF increases migration, proliferation, and collagen production of fibroblasts and regulate inflammation, fibroplasia and remodeling, thus can increase the collagen fibrillogenesis and density during different stages of tendon healing [5,6]. It has a role in migration and proliferation of endothelial cells and therefore can accelerate angiogenesis, short term after injury [94,96,97]. VEFG regulates and increases the migration and proliferation of endothelial cells and both the FGF and VEGF have a strong role in cell differentiation shortly after injury induction and during the initial stages of wound healing [30,95-99]. PDGF is released from the alpha granules of the aggregated and activated platelets after injury and have crucial roles in regulating different stages of healing [30,91,100]. They also enhance activation of macrophages and facilitate the fibroplasia and remodeling at long term [30]. TGF-β has a role in matrix remodeling and increase tissue alignment and density of the collagen fibers during healing process [93]. It also has a role in reducing the tissue metabolism at long term and regulates cell behavior and production at different stages of healing [1,93]. Therefore the role of growth factors in tissue healing and regeneration should not be neglected [30]. Platelet Rich Plasma (PRP) has been shown to have some of these growth factors and has effective value in healing and regeneration [30]. PRP is prepared via the circulating blood using one or two step centrifugation. There are several types of PRP tested in the literature with variable results [30,100]. Several important issues exist with PRP that should be addressed in future investigations. Perhaps, preparation method of PRP, its concentration, source (e.g. auto- allo- and xenogenic base) and its constitutions (platelet or platelet with buffy coat) are suggested to be different between several studies [30,101-103]. As a general rule, effective PRP is prepared via two steps centrifugation with the combination of platelets and buffy coats (inflammatory cells) and has a 6 to 7 times more platelets than the circulating blood [30]. PRP could also be activated to form a gel. It has been suggested that PRP-gel has a higher efficacy in biologic environments because the gel form releases more growth factors during wound healing [30].

In vivo animal based investigations have showed beneficial effects of PRP in tendon and ligament healing while its value in clinical trial studies is questionable [103-106]. This substantial differences has been suggested to be related to the source of PRP [30]. In the animal experiments allogenous PRP have been used while in the clinical studies the autogenous form has been used [30]. Therefore it has been suggested that the effect of PRP may be related to its effectiveness on inflammation in which the allogenous PRP results in more inflammatory reaction than the autogenous PRP [30].

Pro-inflammatory mediators such as Matrix Metalloproteinases (MMPs), Interleukins (IL) and Tumor necrosis factor-α (TNF- α), are important factors which regulate the inflammation phase of healing directly and fibroplasia and remodeling indirectly [1]. These mediators activate different cells and regulate their behavior during healing process and have a role in graft acceptance or rejection [68,70,71]. Th1 lymphocytes produce pro-inflammatory cytokines such as Interleukin-2 (IL-2), Interferon-γ (IFN-γ), and Tumor Necrosis Factor-β (TNF-β) leading to macrophage activation, and can be associated with poor tissue remodeling outcome as well as rejection of both allogenic and xenogenic transplants. On the other hand, Th2 lymphocytes produce IL-4, IL-5, IL-6, and IL-10, cytokines that do not activate macrophages and are probably associated with transplant acceptance [68].

Macrophages are characterized as M1 or M2 phenotypes based on receptor expression, function and production of cytokines. M1 macrophages produce large amounts of pro-inflammatory cytokines such as IL-12 and TNF-α which promote inflammation and express CD68 and CD80 surface markers in rats. On the other hand, M2 macrophages produce large amounts of IL-10 and TGF-β, inhibit release of pro-inflammatory cytokines, promote constructive tissue remodeling and express CD163 surface markers in rats [70]. M2 macrophages induce the Th2 response which is beneficial for the constructive tissue remodeling [70]. The presence of cellular material within an Extracellular Matrix (ECM) scaffold modulates the phenotype of the macrophages and lymphocytes involved in the recipient immunity response after implantation; this can be related to tissue remodeling outcome, acceptance or rejection [29]. Indeed, a cellular graft elicits M1 macrophage and Th1 lymphocyte response,

and can result in the deposition of connective tissue and rejection of the graft [29]. An acellular graft elicits M2 macrophage and Th2 lymphocyte response, leading to more constructive tissue remodeling outcome and acceptance of the graft [68,70,71]. It may be possible that the exogenous pro-inflammatory mediators affect the graft acceptance and rejection *in vivo* [1,12].

Large numbers of healing agents have been extensively tested in the literature with beneficial outcomes [3-11,107-115]. Honey and aloe Vera have been used for skin wound healing and have shown that they can modulate inflammation and increase collagen production and aggregation [110,114]. Tarantula cubensis is a novel therapeutic agent which is commercially available in market as theranekron and has been shown to lyse the necrotic tissues formed after tendon injury thus increases the healing rate [8,11]. Tarantula cubensis has also been shown to regulate different stages of inflammation in order to increase the healing response at further stages of tendon healing [8,11]. Garlic and sylimarin has been shown to be anti-inflammatory and anti-oxidant agents who their effectiveness on wound healing have been extensively described [115,116]. Several other agents such as vitamin C, non-steroidal anti-inflammatory agents such as indomethacin and celecoxib have been shown to regulate the inflammation and are effective in the healing quality [1,117,118]. Piascledine is a novel therapeutic supplement which is produced by a combination of soybean and avocado with a unique proportion and have been shown to increase the collagen production and matrix remodeling [119]. All these agents together with others that have not been listed here could be used in combination with scaffolds, glycosaminoglycans, growth factors and pro inflammatory mediators in order to increase the efficacy of each well designed treatment strategy.

Stem Cells

Stem cells are another category of tissue engineering [32]. Large varieties of stem cells have been differentiated and tested both *in vitro* and *in vivo* [32]. The most well-known stem cells are the mesenchymal types which are differentiated from bone marrow, adipose tissue and blood [120-123]. The bone marrow types have been extensively used in clinical practice but its efficacy is not well described both in clinical trials and animal studies [32,120-123]. Other types of stem cells including pluripotent embryonic stem cells, cord blood stem cells and fetal stem cells are currently under investigation [32]. Cells are the most important factor in wound healing [124-126]. It is a fact that without cells there is no healing response [1]. Endogenous healing cells migrate, proliferate, differentiate and produce matrix during healing process, but the role of exogenous stem cells is in doubt [120-126]. It has been suggested that the effectiveness of stem cells is related to their differentiation state [32]. The higher the differentiated state, the more effective in the healing [32,126]. Based on this statement it has been suggested that the more undifferentiated stem cells have the more capacity to produce tumor or undesirable tissue in the wound [32]. There is also another challenge. The higher differentiated cells generally have lower telomere length and aged during wound healing thus they may not be able to present at later stages of healing in order to be able to produce useful matrix [126]. The most important concern regarding stem cells usage is their viability during the inflammatory phase of tendon and ligament healing [32]. By inflammatory phase, several cytokines and cytotoxic proteins and inflammatory factors are released by the necrotized tissues and inflammatory cells which reduce the viability chance of the stem cells in the host [1,12]. There is no well approved and comprehensive study to answer this question [12]. The results of various investigations are also contraindicated [120-127]. Some studies suggested the effectiveness of various stem cell types including differentiated and undifferentiated ones while others suggest no effectiveness [126-128]. Therefore use of stem cells is only primitive, limited to *in vitro* and animal experimental studies, and thus it could not be suggested as a first line of treatment [30]. If the above concerns are going to be solved one day then the stem cell option could be a new insight in tissue engineering and regenerative medicine [32]. Cell seeding is one of the interesting area in tissue engineering research, it is possible to seed each scaffold with stem cells in order to produce a viable graft similar to autograft [27-29]. This increases the healing incorporative characteristics of the graft and increases the tendon and ligament regeneration capacity [12,32]. Healing promotive factors could be used as a culture medium for stem cells which seeded in the scaffolds [30-32]. Therefore combination of stem cells, healing promotive factors and scaffolds is the newest strategy with the aiming to produce a viable and effective tissue graft [12]. However, despite many advances in the recent years, more investigations are required to confirm this strategy [3].

Gene Therapy

Gene therapy is the final category in tissue engineering and it involves in the transfer of genetic information to target cells, and may introduce safe and effective strategies to induce tissue healing [129-131]. Gene therapy can be used for delivery of growth factors in tissue engineering [130]. The vehicle for gene delivery can be either viral (adenovirus, retrovirus, adeno-associated viruses) or non-viral (liposomes). However, this approach, as others, has a series of limitations, including trans-infection of the target cells with the foreign genes [129]. Furthermore, an unsolved issue of gene therapy is to target the right gene at the right location in the right cells, and express it sufficiently at the right time while minimizing adverse reactions [129,131]. A short controlled expression is desirable and often sufficient to accelerate bone healing, while achieving permanent or long-term expression of a therapeutic gene is more difficult [129]. Therefore, providing controlled and sufficient expression by adaptation of gene therapy to tissue engineering is a key and critical aspect in this field [130].

Tendon and Ligament Healing

Tendons have three different areas including tendon proper which is completely tendinous in nature, musculo-tendinous junction and finally the tendon to bone junction which has three areas of bone, cartilage and tendon [1,12]. Ligaments have two different areas including the ligament part, and bony attachments [3]. In the bony attachment site there are different parts including ligament fibers, sharpey's fibers, cartilage and bone [3,132,133]. Therefore healing of tendons and ligaments are different based on the above mentioned areas [133]. In the tendinous and ligamentous part which are mostly consist of collagen fibers, there are three overlapping healing phases following injury induction [1-3]. These phases are inflammatory, fibroplasia and remodeling [12]. After tendon and ligament injuries, a lag phase is started [1]. In this healing phase, the vessels are disrupted due to injury and the blood enters the injured area [12]. By exposing the circulating blood to the injured collagen fibers, the platelets aggregate and by regulating several mechanisms a blood clot is formed in the wound area [30]. This clot have several roles in the healing process [1,30]. It acts as a scaffold for the inflammatory and mesenchymal cells, thus it creates a tridimensional environment which enables the cells to migrate, attach, proliferate and do their pathophysiological duties [12]. During this phase the platelets release their growth factors and pro inflammatory mediators consisting of mainly PDGF, IGF, FGF and VEGF from their α-granules and the injured cells release

pro inflammatory cytokines which regulate migration and activation of inflammatory cells [1-3,12,24,30]. Neutrophils and macrophages are the first line inflammatory cells which enter and release several growth factors and cytokins in the wound area which accelerate the inflammation and absorb more inflammatory cells in the wound area [132,133].

Transforming growth factor-β (TGF-β) is a product of most cells that are involved in the healing process; its 3 isoforms give rise to distinct spatial responses leading to its diverse effects that regulate several events [93]. During the initial inflammatory phase after trauma, TGF-β expression is elevated and stimulates cellular migration and proliferation, as well as interactions within the repair zone [1]. Synthesis of collagen type I and collagen type III is greatly increased during the later phases [4]. One of the isoforms of the growth factors, TGF-β1, is responsible for the initial scar tissue formation which establishes tissue continuity at the wound site [91]. In the later phases of wound healing, enhanced expression of TGF-β1 leads to scar proliferation and reduced functionality [96]. Transforming growth factor-β3 acts as a negative regulator of scarring at the wound site. Transforming growth factor-β also serves to mechanically regulate the synthesis of collagen in tendon during physical exercise [97].

During the initial repair process and the inflammatory phase, upregulation of growth factors and cytokines such as Insulin-like Growth Factor-1 (IGF-1) stimulate the migration and proliferation of fibroblasts and inflammatory cells to the wound site [91]. Insulin-like growth factor-1 may be stored as an inactive precursor protein in normal tendon and, upon injury, enzymes release the growth factor to exert its biological activity [12]. During the later phases such as remodeling, IGF-1 stimulates synthesis of collagen and other extracellular matrix components. The effects of IGF-1 on matrix metabolism are dose dependent [1]. Cell proliferation and collagen content increase on treatment with IGF-1 [30]. These changes are accompanied by increased stiffness in the treated tendons. Platelet Derived Growth Factor (PDGF) induces the expression of other growth factors such as IGF-1 during the initial repair phase [30]. In addition, the delivery of PDGF to tendon injuries in animal models increases cell proliferation and stimulates the synthesis of collagen and other ECM components in a dose dependent manner during the remodeling phase [30]. Some studies have shown that a phased delivery of PDGF over a longer duration may be desirable to obtain repair that leads to regeneration of a tissue that is functionally closer to normal tendon [95]. Experiments conducted *in vivo* and *in vitro* attest to the mitogenic activity of basic Fibroblast Growth Factor (bFGF), a potent factor that is involved in cell migration and angiogenesis in addition to cell proliferation. Basic fibroblast growth factor has been immunolocalized during tendon repair and is expressed by fibroblasts and inflammatory cells [1,12]. Wound healing models has shown distinctly faster wound closure on treatment with increasing doses of bFGF [5].

Vascular Endothelial Growth Factor (VEGF) is critical for neovascularization, and in the later phases, VEGF is essential in establishment and maintenance of the vasculature present in the endotenon and epitenon [98]. Postoperatively, VEGF expression in healing tendons shows a biphasic expression by majority of the cells within the repair site [30]. Delivery of the bone morphogenetic proteins (BMP) -12, -13, and -14 (also known as Growth/Differentiation Factor [GDF] -7, -6, and -5 or Cartilage Derived Morphogenetic Protein [CDMP] -3, -2, and -1, respectively) to ectopic sites leads to the formation of tendon-like connective tissue [1]. GDFs also increases tendon's callus [30]. Growth/differentiation factors regulate synthesis of the ECM components and expression of collagen type I and collagen type III [1]. These polypeptide factors regulate the expression of specific genes that are found in common among tendons and ligaments, and the expression of these genes serve as markers of tendinogenesis [91-93].

MMPs are important regulators of extracellular matrix network remodeling, and their levels are altered during tendon healing [1]. Expression of the MMP-9 and MMP-13 (collagenase-3) peaks between the first two weeks of injury [12]. MMP-2, MMP-3, and MMP-14 (MT1-MMP) levels increase after the injury and remain high until one month after injury [12]. MMP-9 and MMP-13 participate only in collagen degradation, whereas MMP-2, MMP- 3, and MMP-14 participate both in collagen degradation and in collagen remodeling [1].

The main role of neutrophils is in the acute inflammation is phagocytosis [29]. Macrophages are blood monocytes which migrated from circulating blood through a process called *diapedesis* [12]. Macrophages have more role than phagocytosis and they are the main cells responsible for growth factor and MMPs delivery at the wound site [27-29]. Neutrophils and macrophages phagocytize the necrotic tissues and macrophages regulate important events in the inflammation which is responsible for the transition to fibroplasia of healing as the second stage [25,26]. Macrophages activate the mesenchymal cells' migration, attachment and proliferation in the wound site [4]. At later stages of inflammation the neutrophils decrease in number and the lymphocytes migrate in the wound area thus regulate the macrophage behavior [5]. As suggested Th lymphocytes regulate macrophage response and can determine whether the graft should be accepted or rejected and this completely depends on several important characteristics of the graft which is mainly due to the immunogenicity of the graft [68-72]. When the mesenchymal cells infiltrated in the injured area, the growth factors regulate their behavior thus those cells are in the exposure of bFGF and some other growth factors, are differentiated to fibroblasts and those are mainly exposed to VEGF are differentiated to endothelial cells [5,6,10,12]. Fibroblasts are the main cell types responsible in collagen production while the endothelial cells are responsible for angiogenesis and vessel production [1]. When these cells are differentiated large amounts of glycosaminoglycans (primarily hyaluronic acid), collagen and immature blood vessels are produced in the wound area and at this time the fibroplasia is in progress [7]. Gradually the fibroblasts produce more collagen than glycosaminoglycans and also they produce more polysulphated glycosaminoglycans than hyaluronic acid [12]. As stated, hyaluronic acid is produced at earlier stages of fibroplasia while the polysulphated glycosaminoglycans are produced at later stages of fibroplasia and in the earlier stages of remodeling phase of the healing process [3]. Hyaluronic acid plays an important role in formation of the initial extra cellular matrix thus providing an optimum environment for collagen deposition and cell activity while the polysulphated glycosaminoglycans play important role in collagen aggregation and maturation and regulate collagen fibril diameter and density [12].

The quality of fibroplasia is well correlated with the higher number of fibroblasts and blood vessels [28]. At this moment the immature blood vessels connect to each other and several unconnected immature blood vessels lyse and removed from the healing matrix [29]. The connected blood vessels establish the tissue circulation and nourish the healing fibroblasts to produce more collagenous matrix [12]. In the early fibroplasia the collagen fibers mainly consist of type III which is an immature collagen type while at later stages the matrix consists of mainly of collagen type I which is a mature collagen type [9]. Ultrastructural investigations suggest that the collagen fibrils of type III are disconnected from each other, have low longitudinal and transverse diameter while gradually these collagen fibrils connect each other to produce larger diameter collagen type I fibrils [4-11]. These

fibrils are longer and also have larger transverse diameter at the end of fibroplasia and at remodeling phase of tendon and ligament healing and can transfer the weight bearing forces thus have biomechanical characteristics [7]. By time, the weight bearing forces gradually increase and therefore, the collagen fibers became more aligned along the direction of the stress line that is transmitted between muscle to bone in tendons and between bone to bone in ligaments [12]. By increasing in the exogenous forces and aligning the collagen fibers, the cellularity gradually decreases and tendon metabolism decreases [7-10,134,135]. At this time the remodeling phase of tendon healing which has started by collagen fiber alignment and cell reduction is in progress and gradually moves to maturation and consolidation stages [1]. During the maturation stage of remodeling phase the vascularity gradually decreases, the alignment increases and the metabolism of fibroblasts' decreases and these cells shift from mature fibroblast to highly matured fibrocytes [12]. The fibrocytes have least transverse diameter and the nucleus/cytoplasm ratio is maximum as compared to immature and mature fibroblasts [4,29]. At this stage the collagen fibrils aggregate laterally and pack to form the bundles of collagen fibers [27]. Collagen fibers could be detected under light microscopy and are well responsible for the higher biomechanical properties of the healing tissue [1]. Collagen fibrils have diameter ranging from 30 to 300 nm while the collagen fibers' diameter starts from few to several micrometers [12]. At consolidation stage the endotenon is well developed between the packs of collagen fibers and produce the secondary collagen fiber bundles and fascicles [12]. Collagen differentiation may be terminated at fiber level or may be progressed to produce large fascicles and bundles of collagen fibers [25,134,135]. Consolidation stage of tendon and ligament healing takes several months to years to be terminated and despite of these improvements the biomechanical properties of the healing tendon would never be achieved [1].

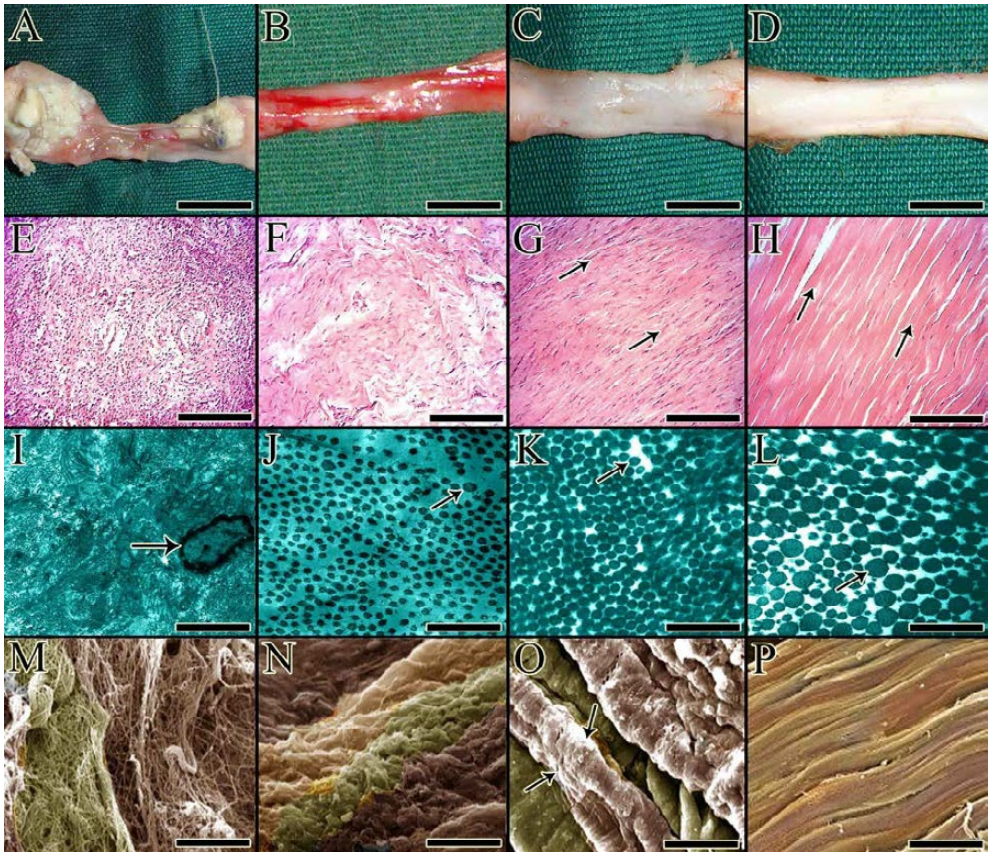

Figure 3: Three different stages of tendon healing under gross morphology, transmission electron-, and scanning electron microscopies.
(A to D) are gross morphologic Figures, (E to H) are light microscopic micrographs, (I to L) are transmission electron ultramicrographs and (M to P) are scanning electron micrographs. (A, E, I and M) show an injured healing tendon in the inflammatory phase of tendon healing. (B, F, J and N) show an injured healing tendon in the fibroplasia phase of tendon healing. (C, G, K and O) show an injured healing tendon in the early remodeling phase of tendon healing. (D, H, I and P) show an injured healing tendon in the late remodeling phase of tendon healing. In the inflammatory phase of tendon healing the injured tendon is inflamed and the tendon edges are necrotized (A). The inflammatory cells consisting of neutrophils, lymphocytes and macrophages are infiltrated in the defect area and start to lyse the necrotic tissues (E). By transmission electron microscopy (I), a macrophage (arrow) could be seen in the injured area and the ultrastructure of the injured area is highly amorphous and no fibrillar pattern could be detected because most of the collagen fibrils of the scaffold have been lysed and no characteristic fibrillogenesis has still occurred. Under scanning electron microscopy the density of the collagen fibrils is much low and most of the tissue spaces between the collagen fibrils are free of collagen fibrils. In fibroplasia, the vascularity of the tissue is high and the injured area of the tendon is hyperemic (B), the collagenous matrix is formed but it is immature in nature because the collagen fibers are not still formed but the number of collagen fibrils is gradually increasing (F). Generally the tissue is hypercellular and the density of the collagenous mass is low (F). At ultrastructural level, the immature collagen fibrils have newly developed (arrow) but their density is low (J). Under scanning electron microscopy (N) the collagen fibrils have developed but they have not still developed to collagen fibers. At early remodeling phase, the hyperemia have reduced and the density of the new tissue has increased but still is not comparable to normal (C). The collagen fibers are recently formed and are unidirectionally aligned (G, arrows). At ultrastructural level, the density and diameter of the collagen fibrils have increased (K) so that the collagen fibers are formed which could be well detected under scanning electron microscopy (O, arrows). In the late remodeling phase, the density of the new tendon have greatly increased (D), and the bundles of collagen fibers are clearly detectable (H) and are highly aligned (H). Under transmission electron microscopy (I) the collagen fibrils are distributed in multimodal pattern so that more than three different categories of collagen fibrils could be detected based on their transverse diameter (I). Under scanning electron microscopy, the collagen fibers that have well differentiated and are densely packed could be seen. Scale bar: A to D: 1 cm, E to H: 50 μm, I to L: 900 nm, M to P: 2 μm.

Two distinct models have been proposed to explain the mechanism of tendon healing including extrinsic and intrinsic mechanisms [1,2,12,29]. In the extrinsic mechanism, tenoblasts and inflammatory cells move from the periphery or external tissue sources to invade the healing site and initiate, and later promote, repair and regeneration [12]. This process includes the initial formation of adhesions and requires a well-established vascular network for the tissue to heal effectively [12]. Intrinsic healing occurs through a mechanism that entails the migration and proliferation of cells from the tendon proper including edges of tendon defect into the injury site; these cells establish an extracellular matrix and an internal neovascular network [1]. In most cases, both mechanisms are involved in the healing phenomenon that is dependent on several factors, including tendon location, extent of trauma, and postoperative motion [7,12]. The extrinsic mechanism, activated earlier than the intrinsic mechanism, is responsible for the formation of adhesions that occur initially, the disorganized collagen matrix with high cellularity, and high water content in the injury site [1,2]. By contrast, the intrinsic mechanism is responsible for the reorganization of the collagen fibers and maintenance of the fibrillar continuity [4,5,12]. It has been suggested that the intrinsic mechanism is the sole responsible for most of the beneficial events that regulates the structure and strength of the new tendon (Figure 3) [12].

Tendon to Bone Healing

As stated tendon and ligaments have bony attachments [3]. Tendon-bone incorporation of a tendon graft within the bone tunnel is a major concern when using a tendon graft for ligament reconstruction [132]. Successful ligament reconstruction with a tendon graft requires solid healing of the tendon graft in the bone tunnels [3]. Improvement of graft healing to bone is crucial to facilitate early and aggressive rehabilitation and a rapid return to full activity [133]. Healing of a tendon graft in a bone tunnel requires bone ingrowth into the tendon [132]. Indirect Sharpey's fibers and direct fibrocartilage fixation of the tendon-bone interface provide different anchorage strength and interface properties [133]. The insertion of the native ligament is characterized in four layers: ligament, fibrocartilage, mineralized fibrocartilage, and bone (Figure 4) [1]. The collagen fibers of the ligament extend into both the fibrocartilage and the mineralized layer [3]. This structure usually is destroyed when the ligament is removed and the bone tunnel is drilled [3]. A replication of this direct type of insertion may be considered desirable when assessing bone tunnel healing for ligament grafts [132]. Based on normal ligament structure and function of the insertion site, the ideal tendon graft would attach broadly to the surface of the bone at the origin and insertion attachment sites by an intermediate zone of fibrocartilage (Figure 4) [3]. The bone-tendon-bone graft undergoes a process of ligamentization [3]. The graft undergoes initial processes of necrosis, revascularization, cellular proliferation, and then remodeling [132,133]. The remodeling phase could be divided into consolidation and maturation phases [1]. The biochemical and morphological changes occur in the graft as it assumes a histologic pattern similar to native ligament, but it is not identical to either native ligament or tendon [3]. The most rapid healing time at the insertion sites is in bone-tendon-bone autograft (e.g. patellar tendon bone autograft) or allograft with bone-to-bone healing times of 4-6 weeks [136]. This graft has the additional benefit of having the natural insertion site of tendon preserved on the bone plug as previously described [3].

The mechanism by which graft-bone healing occurs depends on the type of the graft used. For bone-tendon-bone grafts, healing in the tunnel resembles normal fracture healing but may be a more complex process [3]. Incorporation of the bone block in the tunnel has been observed as early as 16 weeks after surgery [133]. Bone-tendon-bone grafts (e.g. bone- patellar tendon- bone grafts) have the advantage of allowing rigid fixation of the graft in the bone tunnel [133]. The tendon-bone healing process occurs through a different mechanism after implantation of a soft-tissue graft without bone plugs [3]. First, fibrovascular interface tissue forms between graft and bone, and progressive mineralization of the interface tissue occurs with subsequent bone ingrowth into the outer tendon and incorporation of the tendon graft into the surrounding

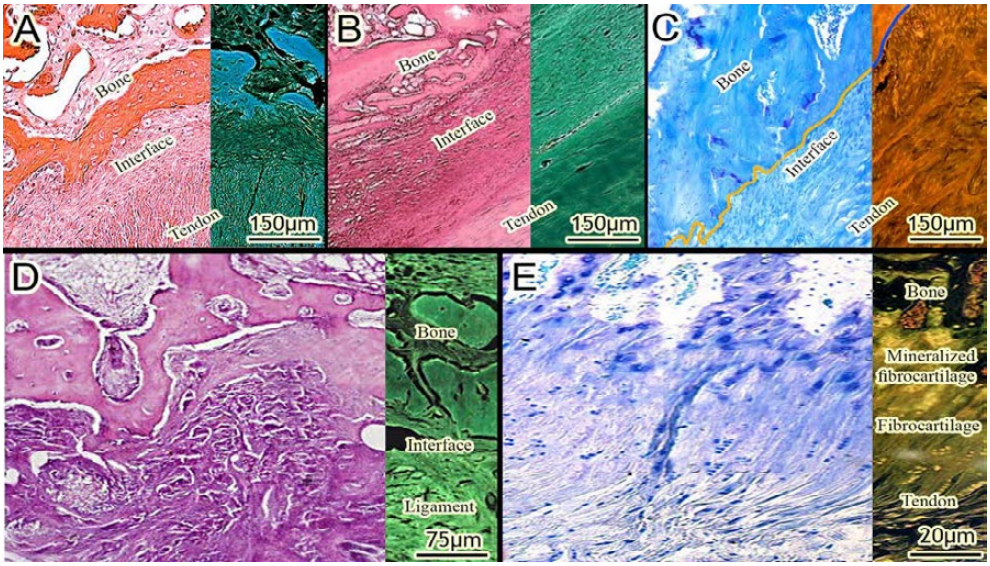

Figure 4: Tendon to bone junction.
The insertion of the native tendon is characterized in four layers: tendon, fibrocartilage, mineralized fibrocartilage, and bone (A to E). The collagen fibers of the tendon extend into both the fibrocartilage and the mineralized layer (E). The tendon-bone healing process occurs as follows: First, fibrovascular interface tissue forms between the graft and bone, and progressive mineralization of the interface tissue occurs with subsequent bone in growth into the outer tendon and incorporation of the tendon graft into the surrounding bone. Sharpey's fibers are made up of type I collagen and connect the periosteum to the bone. Progressive reestablishment of the continuity of collagen fibers between the tendon and the bone results in re-establishment of a tendoosseous junction. Tendoosseous junction is the interface zone (A to D). A and B stained with H and E. C to E stained with toluidine blue.

bone [132,133]. Sharpey's fibers are made up of type I collagen and connect the periosteum to the bone. Progressive re-establishment of the continuity of collagen fibers between the tendon and the bone results in re-establishment of a tendoosseous junction [133]. Formations of the Sharpey-like fibers within the bone tunnel often are identified as a marker of indirect healing between the tendon and bone. Formation of these collagenous fibers may start from 6 weeks after surgery [132]. However, complete bone tunnel healing of a ligament graft may occur as late as 6 to12 months after surgery [136]. It has been postulated that tendon graft incorporation occurs more slowly than bone-tendon-bone healing [3]. In addition to the choice of graft, surgical fixation, graft position, and interfacial motion within the bone tunnel may affect healing [3]. Graft motion within the bone tunnel has been shown to be inversely proportional to healing in animal models [132]. Histology taken at revision surgery for mid-substance tears, shows that free tendon autograft has adequate osteointegration between 6-15 weeks [3]. If soft tissue-to-bone integration occurs at this time, a tendon graft may allow for earlier recovery and return to activity because of less donor site morbidity [12]. A number of studies have shown no significant difference in clinical outcomes between bone-tendon-bone graft and tendon graft for ligament reconstruction [3,132,133]. The healing potential of a ruptured ligament as described before is considered to be extremely poor [12]. It is suggested that the intra-articular environment that inhibits ligament healing also may interfere with bony healing in the proximal parts of the osseous tunnels [3].

Limitations of tendon and ligament healing

Adhesion formation after intrasynovial tendon injury poses a major clinical problem. Disruption of the synovial sheath at the time of the injury or surgery allows granulation tissue and tenocytes from around the tissue to invade the repair site [1,12]. Exogenous cells predominate over endogenous tenocytes, allowing the surrounding tissue to attach to the repair site and resulting in adhesion formation [12]. We showed such limitation happens in the extra synovial flexor tendons healing too [25-29].

As another limitation, despite remodeling the biochemical and mechanical properties of healed tendon never match those of the intact tendon [28]. Finally, healing of large tissue deficits is a complicated process and the healing response and rate is low, resulting in formation of non-functional scar tissue. In tendon to bone junction, dehiscence at the attachment site is one of the most important limitations especially in ligament reconstruction [3].

Host-graft interaction

At least five possible biologic responses have been suggested after implantation of extracellular matrixes including: extracellular matrix nonincorporating responses (ie, encapsulation and rejection) and extracellular matrix incorporating responses (i.e., resorption, integration with progressive degradation, adoption, and adaptation) [29,69]. These responses are dependent to several factors [69]. The best results have been suggested for autografts because their cellular structures are the same as host and also they have least post-implantation immunological reaction [72]. In such condition the inflammation is not triggered by the graft thus there is no rapid absorption [72]. The cellular structures of autografts could well collaborate in the healing process and can produce matrix as the healing fibroblasts do [69-72]. The collagen fibers of autograft are also participate in regeneration mechanism [87,89]. In the other meaning, the host uses most portions of the autograft matrix and therefore there is no need for the host to produce large amounts of collagenous materials and therefore the risk of adhesion formation reduces [87]. Investigations have shown that following autograft implantation the inflammatory cells infiltrate through the graft but they do not accumulate [69-72]. The matrix is partially degraded by phagocytosis and MMPs but most of the matrix is preserved [72]. At fibroplasia the fibroblasts infiltrate the healing graft and the graft is accepted as a part of the new tendon or ligament [12,72]. In this case, the inflammation is minimum and the healing rate is maximum [12]. The autografts have excellent tendoinductivity, tenoconductivity and tenogenisity thus the host behavior following autograft implantation is a combination of integration with progressive degradation, adoption, and adaptation [3]. However the host behavior is different between individuals and species [3,12,29].

Allografts have lower healing capability because most of their cells are not alive; therefore their tendinogenesis properties is lower than the autografts and this leads to inferior healing response to allografts as compared to autografts [3,12,29]. Allografts have two forms including fresh and processed tissues [25-28]. After implantation of a fresh allograft most of its cellular structures are degraded by the apoptosis mechanism and or died and lysed by phagocytosis by the inflammatory cells in the inflammatory phase of healing [69]. As a general rule, the allografts accelerate the inflammatory reaction; therefore more inflammatory cells enter the graft following implantation [12,68]. These cells may accumulate in some areas of the graft to completely lyse the implanted tissue. In the case of fresh allografts the phagocytosis activity of the immune response is much high thus it leads to rapid absorption of the graft [3]. In fresh allograft there is a risk of rejection and the model of rejection is likely to be a rapid absorption [3]. In human and animals models, rejection may occur by different mechanisms [29]. If the graft would be biodegradable such as fresh allografts, then the rejection is activated through the rapid absorption mechanism [69]. If the graft was not biodegradable then the rejection occurs through encapsulation [12]. Therefore application of fresh allograft have not been selected by many surgeons and the processed allograft that have lower antigenicity have been used widely [12,24]. Following implantation of the processed allografts the degree of host inflammatory response is completely dependent on the immunogenicity of the graft and the method of processing [12]. If the antigenicity of the graft was low, then a better outcome could be expected [72]. In that case, the inflammatory is milder and some parts of the graft is persevered and partial matrix replacement occurs by the new tissue [12]. In such situation the processed allograft tissue has no teno- or ligamentoinductivity but has teno- or ligamentoconductivity; it means the graft can act as a scaffold for tissue regeneration and therefore satisfactory results could be expected; however the results are inferior to autografts even with the best outcome [3]. Xenografts are acutely rejected [12,29]. It has been postulated that following xenograft implantation in the tendon defect, the inflammatory cells immediately accumulate in the graft and completely degrade the graft thus only a loose areolar and amorphous tissue replaces the matrix [68-72]. In this case, neutrophils, macrophages and giant cells infiltrate and accumulate in most parts of the graft and degrade it [72]. No acceptable outcome has been reported for the xenografts [72].

As stated, the tissue engineered grafts could be divided into absorbable and non-absorbable ones [25]. Both of the absorbable and non-absorbable materials could be immunogenic i.e. they must be free of immunological characteristics [26]. The host behavior is completely dependent on the above factors [27-29]. If the tissue engineered graft has low immunogenicity and is biodegradable when it is seeded with autogenous stem cells, then it is possible to be accepted as a part of new tendon or ligament similar to the mechanism that was described for autografts [12]. In this case the severity of the inflammation may be

higher than that of the autografts but the duration would not be longer [68,70,71]. These types of tissue engineered cell seeded scaffolds have all the autografts characteristics including tissue inductivity, genesis and conductivity [26]. If the allologous or xenologous cells were seeded in the biodegradable low immunogenic scaffolds, then similar results could be expected as we have described for the fresh allografts and xenografts in which the duration and severity of the inflammation may be prolonged, the graft is acutely absorbed and an amorphous tissue fills the defect [68-72]. Another option is application of scaffolds without stem cells [25-29]. In that case if the scaffold has high immunogenicity and is none resorbable then the scaffold is expected to be rejected by the host through encapsulation mechanism [69]. In fact, fibrous connective tissue covers the scaffold in order to reduce the immunogenic signals and by this mechanism it decreases the duration of the inflammation [12]. In this case the scaffold is preserved in the body but has no function after the healing process [87-89]. If the scaffold was not immunogenic, then it is expected to be preserved and be accepted as a part of the newly developed tissue [25-29]. In that case, the new tissue regenerates through the free spaces of the inner parts of the non-absorbable scaffold but the newly regenerated tissue cannot replace the graft [25]. If the scaffold was not immunogenic but was also biodegradable, an acceptable outcome would be expected and achieved [76,77,85]. Studies suggest that these types of scaffolds would not be completely rejected by the host and some portion of the scaffold may be preserved [28]. In this case some areas of the scaffold may be rapidly infiltrated by the host immune cells and are phagocytized and some remnants parts of the scaffold may be covered by fibrous connective tissue and are encapsulated [27]. It is well shown that following implantation of such bio-absorbable grafts the inflammatory reaction is accelerated but does not last for a long period [25-29]. Therefore, the inflammatory cells consisting of neutrophils and macrophages infiltrate in the scaffold and degrade some parts but rapidly these free spaces are filled with new connective tissue [29].

The portion to be deleted by the host immune response is depended to two factors including the cross linking degree and the immunogenicity of the implant [12]. If the cross linking degree is moderate and the immunogenicity is acceptable, then approximately more than 50% percent of the scaffold is preserved in the fibroplasia stage and numerous small pieces of the implant could be seen as islands of the scaffold. It has been shown that such remnant have major roles in further alignment of the newly developed connective tissue [12,24]. From fibroplasia to the end of healing such remnants could participate in tendon healing and the healing is continued by three major mechanisms [28,29]. In the first mechanism the volume of some of the preserved remnant of the scaffolds gradually decrease but they still act as micro scaffolds and align the new tissue along their longitudinal orientation [28]. In this mechanism the graft remnants are not infiltrated by the fibroblasts and fibrocytes [28]. In the second mechanism the preserved remnants are absorbed faster than the first mechanism [29]. In this mechanism, those free spaces of the implant that were filled with new connective tissue sooner, act as microscaffolds for the newly developed connective tissues that filled the free spaces of the graft later [25]. Therefore, under light microscopy two distinct areas could be seen in the injured area consisting of mature and immature collagen fibers. In the third mechanism the preserved remnant parts are infiltrated by the host fibroblasts and are accepted as parts of the new tendon or ligament [28]. At the remodeling phase most of the implant should be degraded and most of the defect area should be filled with the new but highly aligned tissue (Figure 5) [29].

Postoperative management

The aim of rehabilitation after surgically repaired or reconstructed tendon is to achieve function and gliding but avoiding rupture of the tendon. Although several protocols have been introduced to date, however, the ideal protocol, which gives the best functional outcome, is still under debate.

Historically, patients with Achilles or patellar tendon or ACL rupture are immobilized in a rigid cast for at least 4-6 weeks to allow the presumed tendon healing [137]. Current trends, however, favor minimizing postoperative immobilization and focus on early weight

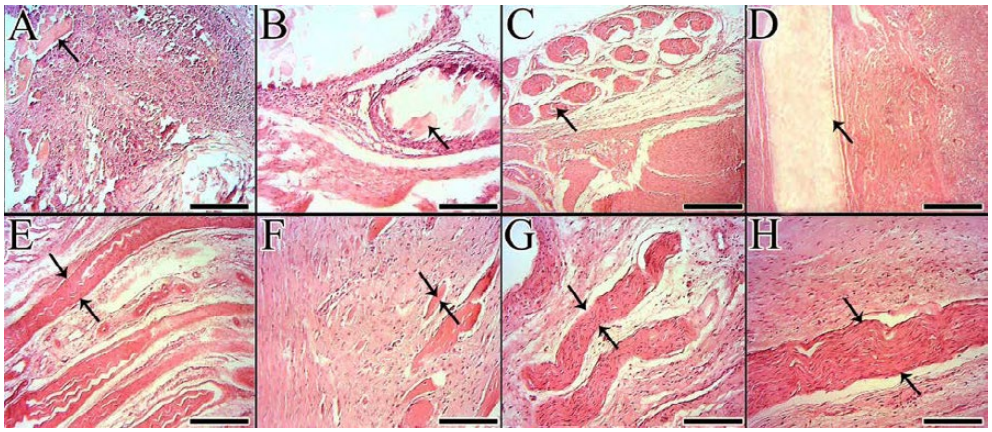

Figure 5: Mechanisms of host graft interactions.
Implantation of various prosthetic implants causes different host behavior based on the nature of the implant. Implantation of the highly antigenic implants cause rapid absorption (A and B) which is described as one of the host rejection mechanisms. Vigorous inflammatory reaction occurs after implantation of the antigenic graft (A) and the inflammatory cells surround the scaffold remnants to completely lyse them at a short period of time. Arrows in A and B show the scaffold remnants. The host can reject the implanted graft by another mechanism which is called encapsulation (C). In fact, the body fails to lyse all parts of the graft, thus the fibrous connective tissue surrounds the graft (C, arrow). If the implant would not be rejected by the host then it can collaborate at different stages of tendon and ligament healing and regeneration by different mechanisms (D to H). D to F is progressive degradation mechanism in which some part of the implant have been absorbed and the new tissue filled the free spaces in the graft and the remnant parts (arrow) are not surrounded by the inflammation and are not encapsulated. The remnant parts are progressively degraded at later stages and the new tissue fills their free spaces (D). The absorption rate in this mechanism is high but is lower than the acute degradation mechanism which we showed in A and B. The final mechanism is adoption and adaptation of the graft (G and H). Some remnants of the graft (arrows) are not rejected by the host, also are not slowly degraded but also infiltrated by the host fibroblasts and accepted as part of the new tendon or ligament. Color staining: H & E. Scale bar for A, C, D, and E: 125 µm and for B, F, G and H: 50 µm.

bearing with excellent results. In a rat model of tendon rupture, using wheel running for early tendon motion, Bring et al. [137] were able to show that physical activity speeds up tendon healing as compared to plaster immobilization. This suggests that there may be some stimulatory effect of mechanical loading on tendon healing. In a clinical prospective study with full postoperative weight bearing in a controlled ankle motion walker accompanied by active stretching, Jacob and Paterson [138] showed few minor complications, high patient satisfaction, and no re-ruptures. Patients with both acute and chronic tears repaired with an open technique did equally well under this treatment protocol. In their meta-analysis of randomized trials comparing early postoperative weight bearing with cast immobilization, Suchak et al. [139] found no difference in re-rupture rates and more excellent rated subjective patient responses. Patients preferred the early function protocol with a mobile cast for multiple reasons including a decrease in leg edema, the ability to bear weight, and easier and faster ability to obtain normal gait after removal of the cast [140].

Here we discussed the rehabilitative protocols of those patients that their hand flexor tendons have been surgically repaired which could be a good example for post-operative rehabilitation program. For hand flexor tendons, there are 3 main methods. Firstly, there is active extension with rubber band flexion, also called the active extension-passive flexion method [141]. Secondly there is passive motion method, which uses a range of 3-5mm of passive motion in the involved tendon and then lastly there is controlled passive motion with the patient actively flexing the digit rather than someone else [141]. There are variations of these methods in clinical practice today. The once popular rubber band technique made known by Kleinert et al., has now been favoured against by passive and active motion protocols [142]. The mobilized tendons have shown to heal quicker and are stronger with lesser adhesions than the immobilized tendons [143]. Programs involve a step-wise progression of passive movement to reduce adhesion but preventing excessive loading and the active motion to overcome stiffness and swelling of the joints. It has been shown that early active protocols and combined Kleinert (passive flexion and active extension) and Durran (controlled passive motion) protocols resulted in low rates of tendon ruptures and acceptable range of motion following flexor tendon repair in zone 2 [144]. Patient compliance is vitally important for success of flexor tendon repairs as patients who negatively ignore the rehabilitation protocols can impact on their results. The trend to more active mobilization seems to be favored but further studies in this area are needed.

The Future

Management of tendon and ligament injuries is technically demanding and the acceptable options are limited [3,12,29]. In addition, tendon and ligament healing and regeneration are complicated processes and have significant limitations including development of post-operative adhesion, low healing response and rejection of the graft [1,12,25]. Autografts are still the gold method; however tissue engineering produced new insights but is still a primitive choice [13-18]. Despite of several achievements in tendon and ligament tissue engineering, the outcome is unclear because the investigations are not organized in order to solve the problems [23,24]. The necessity of animal based studies is felt and it is expected to be done in future in order to better clarify the possible mechanisms and solutions [29]. There are few studies that compared the differences between auto- allo- xeno- and tissue engineered grafts with or without cell seeding methods and other tissue processizations [12,24]. Tissue engineering should answer this question whether is it possible to produce a viable graft alternative to autografts with the same desirable outcome after *in vivo* application. The role of inflammation in tissue healing and regeneration should not be neglected and the possible mechanisms after implantation of various grafts should be better clarified and described [25-29,30-32]. One major problem is that different studies used different methodologies and viable species, thus possible comparison is not logic. It is suggested that the researchers use more standard methods in their investigations and systematic reviews and meta-analyses studies, and comparing the results and concluding their outcomes are highly recommended in future. The role of scaffolds, healing promotive factors including growth factors, glycosaminoglycans, and healing agents, together with stem cells and gene therapy has been discussed in this review. Future investigations should focus on the effectiveness of the combinations of these materials and factors. In designing such novel but interesting tissue engineered based viable bioactive grafts, it is important that the grafts have optimum biomechanical properties at short term and high healing incorporative properties in order to be tissue inductive, -conductive and –genesis at long term. Tissue engineering could open new insights and present a significant achievement in near future and this could be a revolution in healing and regeneration.

Author Contribution

Dr Ali Moshiri and Professor Ahmad Oryan had equal contribution in all parts of the study.

References

1. Sharma P, Maffulli N (2005) Tendon injury and tendinopathy: healing and repair. J Bone Joint Surg Am 87: 187-202.
2. Khanna A, Friel M, Gougoulias N, Longo UG, Maffulli N (2009) Prevention of adhesions in surgery of the flexor tendons of the hand: what is the evidence? Br Med Bull 90: 85-109.
3. Oryan A, Moshiri A, Meimandi-Parizi AH (2013) Graft selection in ACL reconstructive surgery: Past, Present and Future. Current Orthopaedic Practice. Curr Orthop Pract 24: 321-333.
4. Oryan A, Moshiri A, Meimandiparizi AH (2011) Effects of sodium-hyaluronate and glucosamine-chondroitin sulphate on remodeling stage of tenotomized superficial digital flexor tendon in rabbits: a clinical, histopathological, ultrastructural, and biomechanical study. Connect Tissue Res 52: 329-339.
5. Moshiri A, Oryan A (2011) Structural and functional modulation of early healing of full-thickness superficial digital flexor tendon rupture in rabbits by repeated subcutaneous administration of exogenous human recombinant basic fibroblast growth factor. J Foot Ankle Surg 50: 654-662.
6. Oryan A, Moshiri A (2011) A long term study on the role of exogenous human recombinant basic fibroblast growth factor on the superficial digital flexor tendon healing in rabbits. J Musculoskelet Neuronal Interact 11: 185-195.
7. Oryan A, Moshiri A, Meimandi-Parizi AH (2012) Short and long terms healing of the experimentally transverse sectioned tendon in rabbits. Sports Med Arthrosc Rehabil Ther Technol 4: 14.
8. Oryan A, Moshiri A, Raayat AR (2012) Novel application of Theranekron® enhanced the structural and functional performance of the tenotomized tendon in rabbits. Cells Tissues Organs 196: 442-455.
9. Oryan A, Moshiri A, Meimandi Parizi AH, Raayat Jahromi A (2012) Repeated administration of exogenous Sodium-hyaluronate improved tendon healing in an in vivo transection model. J Tissue Viability 21: 88-102.
10. Oryan A, Moshiri A (2012) Recombinant fibroblast growth protein enhances healing ability of experimentally induced tendon injury in vivo. J Tissue Eng Regen Med.
11. Oryan A, Moshiri A, Meimandi Parizi A (2012) Alcoholic extract of tarantula cubensis improves sharp ruptured tendon healing after primary repair in rabbits. Am J Orthop (Belle Mead NJ) 41: 554-560.
12. Moshiri A, Oryan A (2012) Role of tissue engineering in tendon reconstructive surgery and regenerative medicine: current concepts, approaches and concerns. Hard Tissue 1: 11.
13. Beals TC, Severson EP, Kinikini D, Aoki S (2010) Complex Achilles reconstruction for massive soft tissue loss: allograft, autograft, and use of a temporary cement spacer. J Orthop Trauma 24: e78-80.

14. Park YS, Sung KS (2012) Surgical reconstruction of chronic achilles tendon ruptures using various methods. Orthopedics 35: e213-218.

15. Maffulli N, Spiezia F, Testa V, Capasso G, Longo UG, et al. (2012) Free gracilis tendon graft for reconstruction of chronic tears of the Achilles tendon. J Bone Joint Surg Am 94: 906-910.

16. Ronel DN, Newman MI, Gayle LB, Hoffman LA (2004) Recent advances in the reconstruction of complex Achilles tendon defects. Microsurgery 24: 18-23.

17. Bullocks JM, Hickey RM, Basu CB, Hollier LH, Kim JY (2008) Single-stage reconstruction of Achilles tendon injuries and distal lower extremity soft tissue defects with the reverse sural fasciocutaneous flap. J Plast Reconstr Aesthet Surg 61: 566-572.

18. Longo UG, Lamberti A, Maffulli N, Denaro V (2010) Tendon augmentation grafts: a systematic review. Br Med Bull 94: 165-188.

19. Duthon VB, Lübbeke A, Duc SR, Stern R, Assal M (2011) Noninsertional Achilles tendinopathy treated with gastrocnemius lengthening. Foot Ankle Int 32: 375-379.

20. Maheshwari AV, Walters JA, Henshaw RM (2012) Extensile posterior approach to the ankle with detachment of the achilles tendon for oncologic indications. Foot Ankle Int 33: 430-435.

21. Yang M, Wang Z, Li Y, Guo B (2013) Bilateral cadaveric Achilles tendon graft in reconstruction after Achilles tendon tumor resection. J Foot Ankle Surg 52: 103-106.

22. Shearn JT, Kinneberg KR, Dyment NA, Galloway MT, Kenter K, et al. (2011) Tendon tissue engineering: progress, challenges, and translation to the clinic. J Musculoskelet Neuronal Interact 11: 163-173.

23. Chen J, Xu J, Wang A, Zheng M (2009) Scaffolds for tendon and ligament repair: review of the efficacy of commercial products. Expert Rev Med Devices 6: 61-73.

24. Oryan A, Moshiri A, Sharifi P (2012) Advances in injured tendon engineering with emphasis on the role of collagen implants. Hard Tissue 1: 12.

25. Moshiri A, Oryan A, Meimandi Parizi AH, Silver IA, Tanideh N, et al. (2013) Effectiveness of hybridized nano and micro-structure biodegradable and biocompatible collagen-based, three-dimensional bioimplants, in repair of a large tendon-defect model in rabbits. J Tissue Eng Regen Med.

26. Meimandi-Parizi A, Oryan A, Moshiri A (2013) Role of tissue engineered collagen based tridimensional implant on the healing response of the experimentally induced large Achilles tendon defect model in rabbits: a long term study with high clinical relevance. J Biomed Sci 20: 28.

27. Meimandi-Parizi A, Oryan A, Moshiri A, Silver IA (2013) Novel application of a tissue-engineered collagen-based three-dimensional bio-implant in a large tendon defect model: A broad-based study with high value in translational medicine. Tissue Cell 45: 282-294.

28. Moshiri A, Oryan A, MeimandiParizi A (2013) A novel application of biosynthetic tissue-engineered tridimensional implant on large tendon defects: a comprehensive, detailed, in vivo investigation with significant clinical value. Connect Tissue Res 54: 227-243.

29. Moshiri A, Oryan A, Meimandi-Parizi A (2013) Role of tissue-engineered artificial tendon in healing of a large achilles tendon defect model in rabbits. J Am Coll Surg 217: 421-441.

30. Moshiri A, Oryan A (2013) Role of platelet rich plasma in soft and hard connective tissue healing: an evidence based review from basic to clinical application. Hard Tissue 2: 6.

31. Oryan A, Moshiri A (2013) Exogenous acellular synovial fluid as a new therapeutic option in tissue engineering and regenerative medicine. Hard Tissue 2: 21.

32. Moshiri A, Oryan A, Meimandi-Parizi A (2013) Role of stem cell therapy in orthopaedic tissue engineering and regenerative medicine: a comprehensive review of the literature from basic to clinical application. Hard Tissue 2: 31.

33. Sarrafzadehrezaei F, Oryan A, Dehghani S (1999) Effects of electrical stimulation on biomechanics of partial and completed ruptured tendon. Res Med 23: 169-175.

34. Prodromos CC, Han Y, Rogowski J, Joyce B, Shi K (2007) A meta-analysis of the incidence of anterior cruciate ligament tears as a function of gender, sport, and a knee injury-reduction regimen. Arthroscopy 23: 1320-1325.

35. Krackow KA, Thomas SC, Jones LC (1986) A new stitch for ligament-tendon fixation. Brief note. J Bone Joint Surg Am 68: 764-766.

36. Watson TW, Jurist KA, Yang KH, Shen KL (1995) The strength of Achilles tendon repair: an in vitro study of the biomechanical behavior in human cadaver tendons. Foot Ankle Int 16: 191-195.

37. Akgün U, Erol B, Karahan M (2006) [Primary surgical repair with the Krackow technique combined with plantaris tendon augmentation in the treatment of acute Achilles tendon ruptures]. Acta Orthop Traumatol Turc 40: 228-233.

38. Beskin JL, Sanders RA, Hunter SC, Hughston JC (1987) Surgical repair of Achilles tendon ruptures. Am J Sports Med 15: 1-8.

39. Rosenzweig S, Azar FM (2009) Open repair of acute Achilles tendon ruptures. Foot Ankle Clin 14: 699-709.

40. Lindholm A (1959) A new method of operation in subcutaneous rupture of the Achilles tendon. Acta Chir Scand 117: 261-270.

41. Ma GW, Griffith TG (1977) Percutaneous repair of acute closed ruptured achilles tendon: a new technique. Clin Orthop Relat Res: 247-255.

42. Martinelli B (2000) Percutaneous repair of the Achilles tendon in athletes. Bull Hosp Jt Dis 59: 149-152.

43. Davies MS, Solan M (2009) Minimal incision techniques for acute Achilles repair. Foot Ankle Clin 14: 685-697.

44. Thompson J, Baravarian B (2011) Acute and chronic Achilles tendon ruptures in athletes. Clin Podiatr Med Surg 28: 117-135.

45. Winwood PW, Hume PA, Keogh JW, Cronin JB (2013) Retrospective Injury Epidemiology of Strongman Competitors. J Strength Cond Res.

46. Lam KK (2013) Comparative epidemiology of tendon injuries in different racing disciplines. Vet J 195: 265-266.

47. Lopes AD, Hespanhol Júnior LC, Yeung SS, Costa LO (2012) What are the main running-related musculoskeletal injuries? A Systematic Review. Sports Med 42: 891-905.

48. Clegg PD (2012) Musculoskeletal disease and injury, now and in the future. Part 2: Tendon and ligament injuries. Equine Vet J 44: 371-375.

49. Patillo D, Rayan GM (2012) Open extensor tendon injuries: an epidemiologic study. Hand Surg 17: 37-42.

50. Taylor SA, Hannafin JA (2012) Evaluation and management of elbow tendinopathy. Sports Health 4: 384-393.

51. Gajhede-Knudsen M, Ekstrand J, Magnusson H, Maffulli N (2013) Recurrence of Achilles tendon injuries in elite male football players is more common after early return to play: an 11-year follow-up of the UEFA Champions League injury study. Br J Sports Med 47: 763-768.

52. Vosseller JT, Ellis SJ, Levine DS, Kennedy JG, Elliott AJ, et al. (2013) Achilles tendon rupture in women. Foot Ankle Int 34: 49-53.

53. Raikin SM, Garras DN, Krapchev PV (2013) Achilles tendon injuries in a United States population. Foot Ankle Int 34: 475-480.

54. Wilkins R, Bisson LJ (2012) Operative versus nonoperative management of acute Achilles tendon ruptures: a quantitative systematic review of randomized controlled trials. Am J Sports Med 40: 2154-2160.

55. Ackermann PW, Renström P (2012) Tendinopathy in sport. Sports Health 4: 193-201.

56. Joseph MF, Crowley J, Dilieto L, O'Neil B, Denegar CR (2012) Incidence of morphologic changes in asymptomatic Achilles tendons in an active young adult population. J Sport Rehabil 21: 249-252.

57. Swenson DM, Collins CL, Best TM, Flanigan DC, Fields SK, et al. (2013) Epidemiology of knee injuries among U.S. high school athletes, 2005/2006-2010/2011. Med Sci Sports Exerc 45: 462-469.

58. Benner RW, Shelbourne KD, Urch SE, Lazarus D (2012) Tear patterns, surgical repair, and clinical outcomes of patellar tendon ruptures after anterior cruciate ligament reconstruction with a bone-patellar tendon-bone autograft. Am J Sports Med 40: 1834-1841.

59. Weiss JM, Arkader A, Wells LM, Ganley TJ (2013) Rotator cuff injuries in adolescent athletes. J Pediatr Orthop B 22: 133-137.

60. Horsley IG, Fowler EM, Rolf CG (2013) Shoulder injuries in professional rugby: a retrospective analysis. J Orthop Surg Res 8: 9.

61. Mall NA, Lee AS, Chahal J, Sherman SL, Romeo AA, et al. (2013) An evidenced-based examination of the epidemiology and outcomes of traumatic rotator cuff tears. Arthroscopy 29: 366-376.

62. Tekavec E, Jöud A, Rittner R, Mikoczy Z, Nordander C, et al. (2012) Population-based consultation patterns in patients with shoulder pain diagnoses. BMC Musculoskelet Disord 13: 238.

63. Tashjian RZ (2012) Epidemiology, natural history, and indications for treatment of rotator cuff tears. Clin Sports Med 31: 589-604.

64. Starr HM, Snoddy M, Hammond KE, Seiler JG 3rd (2013) Flexor tendon repair rehabilitation protocols: a systematic review. J Hand Surg Am 38: 1712-1717.

65. McCarthy MM, Voos JE, Nguyen JT, Callahan L, Hannafin JA (2013) Injury profile in elite female basketball athletes at the Women's National Basketball Association combine. Am J Sports Med 41: 645-651.

66. Sri-Ram K, Salmon LJ, Pinczewski LA, Roe JP (2013) The incidence of secondary pathology after anterior cruciate ligament rupture in 5086 patients requiring ligament reconstruction. Bone Joint J 95-95B: 59-64.

67. Goddard M, Bowman N, Salmon LJ, Waller A, Roe JP, et al. (2013) Endoscopic anterior cruciate ligament reconstruction in children using living donor hamstring tendon allografts. Am J Sports Med 41: 567-574.

68. Badylak SF, Valentin JE, Ravindra AK, McCabe GP, Stewart-Akers AM (2008) Macrophage phenotype as a determinant of biologic scaffold remodeling. Tissue Eng Part A 14: 1835-1842.

69. Cornwell KG, Landsman A, James KS (2009) Extracellular matrix biomaterials for soft tissue repair. Clin Podiatr Med Surg 26: 507-523.

70. Allman AJ, McPherson TB, Badylak SF, Merrill LC, Kallakury B, et al. (2001) Xenogeneic extracellular matrix grafts elicit a TH2-restricted immune response. Transplantation 71: 1631-1640.

71. Palmer EM, Beilfuss BA, Nagai T, Semnani RT, Badylak SF, et al. (2002) Human helper T cell activation and differentiation is suppressed by porcine small intestinal submucosa. Tissue Eng 8: 893-900.

72. Veillette CJ, Cunningham KD, Hart DA, Fritzler MJ, Frank CB (1998) Localization and characterization of porcine patellar tendon xenograft antigens in a rabbit model of medial collateral ligament replacement. Transplantation 65: 486-493.

73. Pridgen BC, Woon CY, Kim M, Thorfinn J, Lindsey D, et al. (2011) Flexor tendon tissue engineering: acellularization of human flexor tendons with preservation of biomechanical properties and biocompatibility. Tissue Eng Part C Methods 17: 819-828.

74. Fini M, Torricelli P, Giavaresi G, Rotini R, Castagna A, et al. (2007) In vitro study comparing two collageneous membranes in view of their clinical application for rotator cuff tendon regeneration. J Orthop Res 25: 98-107.

75. T Shu-Hua Teng, Eun-Jung Lee, Peng Wang, Hyoun-Ee Kim (2008) Collagen/hydroxyapatite composite nanofibers by electrospinning. Mat Let 62: 3055-3058.

76. Postlethwaite AE, Seyer JM, Kang AH (1978) Chemotactic attraction of human fibroblasts to type I, II, and III collagens and collagen-derived peptides. Proc Natl Acad Sci U S A 75: 871-875.

77. Ghanaati S, Schlee M, Webber MJ, Willershausen I, Barbeck M, et al. (2011) Evaluation of the tissue reaction to a new bilayered collagen matrix in vivo and its translation to the clinic. Biomed Mater 6: 015010.

78. Tsai S, Hsieh C, Hsieh C, Wang D, Huang LL, et al. (2007) Preparation and Cell Compatibility Evaluation of Chitosan/Collagen Composite Scaffolds Using Amino Acids as Crosslinking Bridges. J Appl Polym Sci 105: 1774-1785.

79. Foltran I, Foresti E, Parma B, Sabatino P, Norberto R (2008) Novel Biologically Inspired Collagen Nanofibers Reconstituted By Electrospinning Method. Macromol Symp 269: 111-118.

80. Wray LS, Orwin EJ (2009) Recreating the microenvironment of the native cornea for tissue engineering applications. Tissue Eng Part A 15: 1463-1472.

81. Dubey N, Letourneau PC, Tranquillo RT (2001) Neuronal contact guidance in magnetically aligned fibrin gels: effect of variation in gel mechano-structural properties. Biomaterials 22: 1065-1075.

82. McCall AS, Kraft S, Edelhauser HF, Kidder GW, Lundquist RR, et al. (2010) Mechanisms of corneal tissue cross-linking in response to treatment with topical riboflavin and long-wavelength ultraviolet radiation (UVA). Invest Ophthalmol Vis Sci 51: 129-138.

83. Stillaert FB, Di Bartolo C, Hunt JA, Rhodes NP, Tognana E, et al. (2008) Human clinical experience with adipose precursor cells seeded on hyaluronic acid-based spongy scaffolds. Biomaterials 29: 3953-3959.

84. Siritientong T, Srichana T, Aramwit P (2011) The effect of sterilization methods on the physical properties of silk sericin scaffolds. AAPS PharmSciTech 12: 771-781.

85. Gigante A, Cesari E, Busilacchi A, Manzotti S, Kyriakidou K, et al. (2009) Collagen I membranes for tendon repair: effect of collagen fiber orientation on cell behavior. J Orthop Res 27: 826-832.

86. Ping Ooi C, Cameron RE (2002) The hydrolytic degradation of polydioxanone (PDSII) sutures. Part II: Micromechanisms of deformation. J Biomed Mater Res 63: 291-298.

87. Oryan A (1995) Role of collagen in soft connective tissue wound healing. Transplant Proc 27: 2759-2761.

88. Oryan A (1995) Induction of soft connective tissue formation by various prosthetic implants. Transplant Proc 27: 2754-2755.

89. Oryan A, Moshiri A (2013) Tissue Engineering: Alternative Option in Managing Large and Massive Tissue Deficits. Tropical Medicine & Surgery 1:e101.

90. Oryan A, Goodship AE, Silver IA (2008) Response of a collagenase-induced tendon injury to treatment with a polysulphated glycosaminoglycan (Adequan). Connect Tissue Res 49: 351-360.

91. Barnes GL, Kostenuik PJ, Gerstenfeld LC, Einhorn TA (1999) Growth factor regulation of fracture repair. J Bone Miner Res 14: 1805-1815.

92. Wang JS (1996) Basic fibroblast growth factor for stimulation of bone formation in osteoinductive or conductive implants. Acta Orthop Scand Suppl 269: 1-33.

93. Rosier RN, O'Keefe RJ, Hicks DG (1998) The potential role of transforming growth factor beta in fracture healing. Clin Orthop Relat Res: S294-300.

94. Friesel RE, Maciag T (1995) Molecular mechanisms of angiogenesis: fibroblast growth factor signal transduction. FASEB J 9: 919-925.

95. Canalis E, McCarthy TL, Centrella M (1989) Effects of platelet-derived growth factor on bone formation in vitro. J Cell Physiol 140: 530-537.

96. Steenfos HH (1994) Growth factors and wound healing. Scand J Plast Reconstr Surg Hand Surg 28: 95-105.

97. Lieberman JR, Daluiski A, Einhorn TA (2002) The role of growth factors in the repair of bone. Biology and clinical applications. J Bone Joint Surg Am 84-84A: 1032-44.

98. Peng H, Wright V, Usas A, Gearhart B, Shen HC, et al. (2002) Synergistic enhancement of bone formation and healing by stem cell-expressed VEGF and bone morphogenetic protein-4. J Clin Invest 110: 751-759.

99. Hom DB, Maisel RH (1992) Angiogenic growth factors: their effects and potential in soft tissue wound healing. Ann Otol Rhinol Laryngol 101: 349-354.

100. Tohidnezhad M, Varoga D, Wruck CJ, Brandenburg LO, Seekamp A, et al. (2011) Platelet-released growth factors can accelerate tenocyte proliferation and activate the anti-oxidant response element. Histochem Cell Biol 135: 453-460.

101. Muto T, Kokubu T, Mifune Y, Sakata R, Nagura I, et al. (2013) Platelet-rich plasma protects rotator cuff-derived cells from the deleterious effects of triamcinolone acetonide. J Orthop Res 31: 976-982.

102. de Jonge S, de Vos RJ, Weir A, van Schie HT, Bierma-Zeinstra SM, et al. (2011) One-year follow-up of platelet-rich plasma treatment in chronic Achilles tendinopathy: a double-blind randomized placebo-controlled trial. Am J Sports Med 39: 1623-1629.

103. Oryan A, Meimandi-Parizi AH, Shafiei-Sarvestani Z, Bigham AS (2011) Effects of combined hydroxyapatite and human platelet rich plasma on bone healing in rabbit model: radiological, macroscopical, histopathological, ultrastructural and biomechanical studies. Cell Tissue Bank 13: 639-651.

104. Aspenberg P, Virchenko O (2004) Platelet concentrate injection improves Achilles tendon repair in rats. Acta Orthop Scand 75: 93-99.

105. Wnuk T, Blacha J, Mazurkiewicz T, Olchowik G, Chyżyńska M (2012) The mechanical and histological estimation of calcaneal tendon callus in rats after PRP injection. Pol Orthop Traumatol 77: 5-9.

106. Kaux JF, Drion PV, Colige A, Pascon F, Libertiaux V, et al. (2012) Effects of platelet-rich plasma (PRP) on the healing of Achilles tendons of rats. Wound Repair Regen 20: 748-756.

107. Oryan A, Silver IA, Goodship AE (2010) Metrenperone enhances collagen turnover and remodeling in the early stages of healing of tendon injury in rabbit. Arch Orthop Trauma Surg 130: 1451-1457.

108. Oryan A, Silver IA, Goodship AE (2009) Effects of a serotonin S2-receptor blocker on healing of acute and chronic tendon injuries. J Invest Surg 22: 246-255.

109. Naeini AT, Oryan A, Nikahval B, Toosinezhad M (2012) Evaluation of Glycyrrhiza glabra (Licorice) on wound healing in rats. Online J Vet Res 16: 138-146.

110. Oryan A, Tabatabaeinaeini A, Nikahval B, Gorgian E (2010) Effects of aqueous extract of Aloe vera on experimental cutaneous wound healing in rat. Vet Arhiv 80: 509-522.

111. Tabatabaei AT, Oryan A, Dehghaninazhvani S, Nikahval B (2008) Effects of topical and parentral application of Phenytoin on cutaneous wound healing in rabbit. J Ani Vet Adv 7: 1537-1545.

112. Tabatabaei AT, Oryan A, Dehghani S, Nikahval B (2008) Experimental cutaneous wound healing in rabbits: Using continuous microamperage low voltage electrical stimulation. Comp Clin Pathol 17: 203-210.

113. Oryan A, Khalafi-Nezhad A, Toloo N, Soltani Rad MN (2007) Effects of 4-chloro-2,6-bis-(2-hydroxyl-benzyl)-phenol on healing of skin wounds and growth of bacteria. J Vet Med A Physiol Pathol Clin Med 54: 585-591.

114. Oryan A, Zaker SR (1998) Effects of topical application of honey on cutaneous wound healing in rabbits. Zentralbl Veterinarmed A 45: 181-188.

115. Oryan A, Tabatabaei Naeini A, Moshiri A, Mohammadalipour A, Tabandeh MR (2012) Modulation of cutaneous wound healing by silymarin in rats. J Wound Care 21: 457-464.

116. Nidadavolu P, Amor W, Tran PL, Dertien J, Colmer-Hamood JA, et al. (2012) Garlic ointment inhibits biofilm formation by bacterial pathogens from burn wounds. J Med Microbiol 61: 662-671.

117. Hung LK, Fu SC, Lee YW, Mok TY, Chan KM (2013) Local vitamin-C injection reduced tendon adhesion in a chicken model of flexor digitorum profundus tendon injury. J Bone Joint Surg Am 95: e41.

118. Pavelka K, Coste P, Géher P, Krejci G (2010) Efficacy and safety of piascledine 300 versus chondroitin sulfate in a 6 months treatment plus 2 months observation in patients with osteoarthritis of the knee. Clin Rheumatol 29: 659-670.

119. Ai J, Ebrahimi S, Khoshzaban A, Jafarzadeh Kashi TS, Mehrabani D (2012) Tissue engineering using human mineralized bone xenograft and bone marrow mesenchymal stem cells allograft in healing of tibial fracture of experimental rabbit model. Iran Red Crescent Med J 14: 96-103.

120. Al Faqeh H, Nor Hamdan BM, Chen HC, Aminuddin BS, Ruszymah BH (2012) The potential of intra-articular injection of chondrogenic-induced bone marrow stem cells to retard the progression of osteoarthritis in a sheep model. Exp Gerontol 47: 458-464.

121. Behfar M, Sarrafzadeh-Rezaei F, Hobbenaghi R, Delirezh N, Dalir-Naghadeh B (2012) Enhanced mechanical properties of rabbit flexor tendons in response to intratendinous injection of adipose derived stromal vascular fraction. Curr Stem Cell Res Ther 7: 173-178.

122. Behnia H, Khojasteh A, Soleimani M, Tehranchi A, Atashi A (2012) Repair of alveolar cleft defect with mesenchymal stem cells and platelet derived growth factors: a preliminary report. J Craniomaxillofac Surg 40: 2-7.

123. Cheung WH, Chin WC, Wei FY, Li G, Leung KS (2013) Applications of exogenous mesenchymal stem cells and low intensity pulsed ultrasound enhance fracture healing in rat model. Ultrasound Med Biol 39: 117-125.

124. Chong AK, Ang AD, Goh JC, Hui JH, Lim AY, et al. (2007) Bone marrow-derived mesenchymal stem cells influence early tendon-healing in a rabbit achilles tendon model. J Bone Joint Surg Am 89: 74-81.

125. Clarke AW, Alyas F, Morris T, Robertson CJ, Bell J, et al. (2011) Skin-derived tenocyte-like cells for the treatment of patellar tendinopathy. Am J Sports Med 39: 614-623.

126. Pascual-Garrido C, Rolón A, Makino A (2012) Treatment of chronic patellar tendinopathy with autologous bone marrow stem cells: a 5-year-followup. Stem Cells Int 2012: 953510.

127. Silva A, Sampaio R, Fernandes R, Pinto E (2012) Is there a role for adult non-cultivated bone marrow stem cells in ACL reconstruction? Knee Surg Sports Traumatol Arthrosc.

128. Egermann M, Lill CA, Griesbeck K, Evans CH, Robbins PD, et al. (2006) Effect of BMP-2 gene transfer on bone healing in sheep. Gene Ther 13: 1290-1299.

129. Schwabe P, Greiner S, Ganzert R, Eberhart J, Dähn K, et al. (2012) Effect of a novel nonviral gene delivery of BMP-2 on bone healing. ScientificWorldJournal 2012: 560142.

130. Heyde M, Partridge KA, Oreffo RO, Howdle SM, Shakesheff KM, et al. (2007) Gene therapy used for tissue engineering applications. J Pharm Pharmacol 59: 329-350.

131. Lui P, Zhang P, Chan K, Qin L (2010) Biology and augmentation of tendon-bone insertion repair. J Orthop Surg Res 5: 59.

132. James R, Kesturu G, Balian G, Chhabra AB (2008) Tendon: biology, biomechanics, repair, growth factors, and evolving treatment options. J Hand Surg Am 33: 102-112.

133. Oryan A, Shoushtari AH (2008) Histology and ultrastructure of the developing superficial digital flexor tendon in rabbits. Anat Histol Embryol 37: 134-140.

134. Oryan A, Shoushtari AH (2009) Biomechanical properties and dry weight content of the developing superficial digitalflexor tendon in rabbits. Comp Clin Pathol 18: 131-137.

135. Oryan A, Alidadi S, Moshiri A (2013) Current concerns regarding healing of bone defects. Hard Tissue 2: 13.

136. Metzl JA, Ahmad CS, Levine WN (2008) The ruptured Achilles tendon: operative and non-operative treatment options. Curr Rev Musculoskelet Med 1: 161-164.

137. Bring DK, Kreicbergs A, Renstrom PA, Ackermann PW (2007) Physical activity modulates nerve plasticity and stimulates repair after Achilles tendon rupture. J Orthop Res 25: 164-172.

138. Jacob KM, Paterson R (2007) Surgical repair followed by functional rehabilitation for acute and chronic achilles tendon injuries: excellent functional results, patient satisfaction and no reruptures. ANZ J Surg 77: 287-291.

139. Suchak AA, Spooner C, Reid DC, Jomha NM (2006) Postoperative rehabilitation protocols for Achilles tendon ruptures: a meta-analysis. Clin Orthop Relat Res 445: 216-221.

140. Boyer MI, Strickland JW, Engles DR, Sachar K, Leversedge FJ (2002) Flexor tendon repair and rehabilitation, state of the art in 2002. J Bone Joint Surg Am 84: 1684-1706.

141. Small JO, Brennen MD, Colville J (1989) Early active mobilisation following flexor tendon repair in zone 2. J Hand Surg Br 14: 383-391.

142. Kleinert HE, Kutz JE, Atasoy E, Stormo A (1973) Primary repair of flexor tendons. Orthop Clin North Am 4: 865-876.

143. Tang JB (2007) Indications, methods, postoperative motion and outcome evaluation of primary flexor tendon repairs in Zone 2. J Hand Surg Eur Vol 32: 118-129.

144. Chesney A, Chauhan A, Kattan A, Farrokhyar F, Thoma A (2011) Systematic review of flexor tendon rehabilitation protocols in zone II of the hand. Plast Reconstr Surg 127: 1583-1592.