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Regeneration techniques for bone-to-tendon and muscle-to-tendon interfaces reconstruction

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Abstract

Introduction: The complex structure of the bone–tendon and muscle–tendon junctions makes their reproduction for tissue engineering applications very difficult. Relatively few studies have investigated the characteristics of these regions from a tissue engineering view point.

Sources of data: PubMed, Thomson Reuters, Scopus and Google Scholar databases were searched using various combinations of the keywords 'Tendon', 'Myotendinous junction', 'Osteotendinous junction', 'Tissue engineering' and 'Scaffold'.

Areas of agreement: The available studies can be divided according to whether the objective is to build an entire composite tissue unit or to assist the recreation of interfaces, such as improving integration of autografts with the surrounding bone or with the muscle. The most used techniques are based on the electrospinning and the self-reorganized constructs process, which were applied to both bone-to-tendon junction (BTJ) and muscle-to-tendon junction (MTJ) regeneration. The use of nanofibers that mimic the hierarchical structure of the extracellular matrix (ECM), eventually functionalized by encapsulation of bioactive components, allowed cell attachment and differentiation.

Areas of controversy: There have been no translational investigations.

Growing points: There is a need to devise suitable techniques that allow suitable tissue engineering of BTJ and MTJ.

Areas timely for developing research: Appropriately planned studies are needed to translate tissue engineering from a scientific challenge to a clinically applicable technique.

Key words: tendon, bone–tendon junction, muscle–tendon junction, tissue engineering, scaffold

Introduction

Tendons are highly organized connective tissues that transmit force between muscle and bone.[1] They are characterized by three specialized regions along their whole length: the myotendinous junction (MTJ), the tendon proper with the region where tendons change direction by wrapping around bony pulleys and the bone–tendon junction (BTJ),[2] as shown in Fig. 1.

Therefore, in their function as transducers of the force produced by muscle contraction on the bone around a joint, tendons are interposed between these two histologically and mechanically widely different tissues. Moreover, a muscle transition to a tendon, and a tendon will eventually transition to bone. The MTJ and BTJ are extremely specialized tissues, and, in case of injury at these levels, repair will take place only through mechanically and histologically suboptimal scar tissue. This leads to decreased functional properties of these injured regions and to a greater risk of recurrent injury.

Tendons are resilient during the development of tension, but flexible enough to conform to their mechanically demanding environment. The mechanical performance of tendons depends on the parallel fibrils of collagen, which form the structure. In the resting state, the collagen fibrils exhibit a wavy conformation, defined as crimp. As a tendon is stretched, the crimped collagen fibrils begin to straighten out, and, as a result, the tendon becomes stiffer with increasing application of mechanical strain.[3] Collagen fibrils are organized in fiber bundles, according to a hierarchical structure; the transition from tendon to bone and from tendon to muscle is progressive to allow an efficient load transfer minimizing stress concentrations.[4] The tensile modulus of tendons is ~0.4 GPa in the direction of muscle force during physiological loading conditions;[5] in bone, the tensile modulus is ~20 GPa.[6] Typically, tendon is stronger than its muscle and its bony insertion; thus, it is more common for the MTJ or BTJ to fail before the tendon during overload.[1]

There are ~2 million Achilles tendon sports-related injuries each year worldwide. Of these, over 250 000 require surgical intervention and prolonged rehabilitation. However, patients with these injuries seldom regain full pre-injury function. These ailments are difficult to manage, frequently resulting in long-term pain and discomfort, placing long-term burden on health care. The poor repair of tendons is a direct consequence of their limited vascularity and relatively acellular nature. Most frequently injured are the rotator cuff, the patellar and the Achilles tendons, with pathology ranging from tendinopathy and calcific tendinopathy, to partial tears,

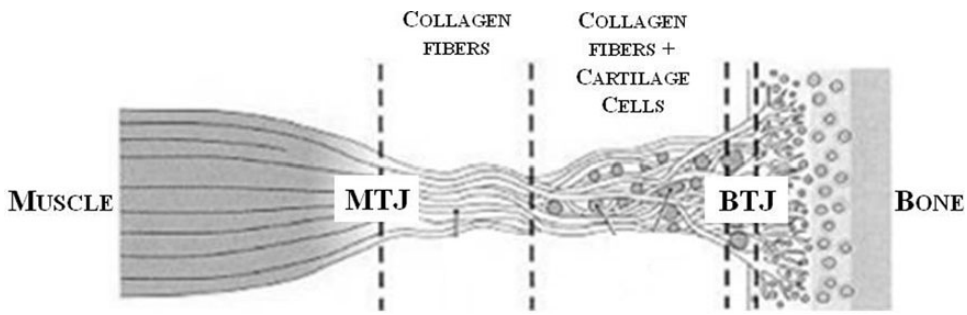

Fig. 1 Picture depicting tendon structure and the junctions at muscle and bone (adapted from Human Anatomy Pt 514 with Salem at University of Southern California).

to complete ruptures. The inability of a tendon to self-repair and the general inefficiencies of current treatment regimes suggest that the identification of alternative strategies is a priority. Other tendon injuries occur at the BTJ. Considering rotator cuff injuries, even accurate, early repair using state of the art techniques results in a relatively high rate of failed reproduction of the BTJ and clinical failure.[7] This has resulted in attempts to enhance healing at the bone–tendon interface, which have included the use of, for example, pulsed electromagnetic fields.[7] Reproduction of a functional BTJ is a necessity in anterior cruciate ligament reconstruction. Again, in this field, biological interventions have been tried, with dubious results in human clinical practice, although animal work seems promising.[8,9] Also muscle injuries are highly prevalent in sports, accounting for 10–55% of all acute sports injuries[10] and are difficult to manage.[11] They occur most frequently at the MTJ, have a high rate of recurrence and are a major cause of permanent inability to continue with sports activities.[12]

The ideal scaffold to be used in tendon tissue engineering (TTE) should be biocompatible, highly porous (with a minimum pore size between 100 and 150 μm)[13] and biodegradable; moreover, it should present other specific characteristics, such as superior mechanical properties and maintenance of mechanical strength during the tissue regeneration process. For example, for rotator cuff tendon repair, scaffolds should possess a stiffness higher than 200 MPa and ultimate load higher than 800 N, as well as a suture retention strength higher than 200 N.[14] In the case of MTJ, it is necessary to develop a scaffold that should possess both a compliant/high strain region (modulus values ranging from 0.012 to 2.8 MPa), a stiff/low strain region (tensile loading values of 500–1850 MPa) and an intermediate region.[15] Moreover, suitable 3-D structures (with a gradient of morphological properties) and shapes are necessary. From a biological point of view, scaffolds should promote cell attachment and migration, and provide an environment suitable for cell proliferation and differentiation, allowing the cells to secrete their own extracellular matrix to form tissue-like organization. All these phenomena should occur within appropriate times (typically, 6–36 months),[16] while the scaffold degrades in a controlled fashion.[17] An overview of the scientific literature on TTE shows that much work has focused on engineering the tendon proper;[18–20] however, given their more complex structure, experimental works on BTJ and MTJ are relatively scarce.

The aim of this review is to organize and critically discuss the relevant articles, published mainly in the period 2000–15, regarding BTJ and MTJ tissue engineering, giving indications about the most promising approaches in this field.

Methods

In this work, a critical review of the literature was performed. The search was based on PubMed, Thomson Reuters, Scopus and Google Scholar databases, using various combinations of the keywords 'Tendon', 'Myotendinous junction', 'Osteotendinous junction', 'Tissue engineering' and 'Scaffold', considering the articles (~100) in the time range from 2000 to 2015. Nevertheless, some interesting papers before 2000 were also included (~15).

All scientific journals were considered and relevant studies were analyzed. The articles were initially screened according to their title and abstract; then, the full-text of each article was downloaded, allowing the investigators to define the relevance of the work. A cross-reference search of the selected articles was also performed to obtain other articles pertinent to the study at hand. The search was performed in May, 2015.

The selected articles, the relative list of references and the articles excluded from the study were reviewed, assessed and discussed by the authors, and, if there was disagreement among investigators regarding the inclusion and exclusion criteria, the senior investigator made the final decision.

Results and discussion

Bone–tendon junction

The morphological structure specific of the BTJ is essential for an efficient transmission of force between soft tissue (ligament, tendon and joint

capsule) and hard bone. Four zones are traditionally described for the tendon-to-bone insertion.[21] Zone 1, 'tendon': it is characterized by 90% v/v of well-aligned collagen fibers, predominantly type I, with small amounts of the proteoglycan decorin and the remaining volume primarily by water. Zone 2, 'fibrocartilage': it contains collagen types II and III, with small amounts of types I, IX and X, and of the proteoglycans aggrecan and decorin.[22,23] Zone 3, 'mineralized fibrocartilage': it is the 'tidemark', interpreted as a mineralization front and a boundary between soft and hard tissue.[21] Zone 4, 'bone': it is characterized by 40% v/v of type I collagen, following the hierarchical structure of tendon, and by 50% v/v of a stiff carbonated mineral, apatite.[24,25]

From a mechanical point of view, Zones 2 and 3 are approximately half as stiff as tendon (Zone 1); bone (Zone 4) is nearly two orders of magnitude stiffer than tendon.[23,26] A sharp transition between tendon and bone would lead to stress concentration and increase the risk of failure at the interfaces. This challenge is well described by the concept of 'functional grading'; i.e. the gradual variation in composition and structure over a given volume, resulting in modifications of the mechanical properties of the material, to relieve stress concentration.[27] As a result of this mechanically efficient structure, traumatic failure of ligaments and tendons normally occurs within the bone or the ligament/tendon substance, away from the insertion, and, more rarely, at the tendon-bone junction itself.[28] Moffat *et al.* determined, using uniaxial microcompression testing combined with video microscopy and optimized Digital Image Correlation (DIC) analysis, the compressive mechanical properties of the anterior cruciate ligament (ACL)-to-bone interface. They observed that uniaxial unconfined compression determined a nonlinear axial deformation across the interface, specifically from the nonmineralized fibrocartilage (NFC) to the mineralized fibrocartilage (MFC), and, then, to the bone region. NFC exhibited greater deformation than the MFC region at all applied strains. Moreover, the apparent Young modulus was significantly greater for the calcified than for the noncalcified interface region. Summarizing, under applied compression, a gradual decrease in insertion site strain is found progressing from the NFC to MFC and then to the bone region, accompanied by a corresponding increase in apparent Young modulus across the interface. This mechanical inhomogeneity minimizes the formation of areas of stress concentration and promotes gradual load transfer from soft to hard tissues.[29]

Muscle–tendon junction

The mechanical utilization of contractile force produced by myofilaments requires that they should be efficiently connected to tendon fibers. This connection between intracellular and extracellular proteins occurs at the MTJ, a specialized region between skeletal muscle fibers and the tendon.[30] The MTJ links cells of different embryological origins; myogenic cells forming the striated skeletal muscles of the limb, which originate from somites, and tendons, which originate from the lateral plate.[31]

MTJ is formed by four separate ultrastructural domains connecting the actin filaments of the terminal sarcomere with the collagen fibers of the tendon. These domains are (i) 'the internal lamina', composed of actin filaments and associated cross-linking structures; (ii) 'the connecting domain', which connects the internal lamina to the external lamina; (iii) 'the lamina densa of the external lamina', with a structure similar to other laminae densae; and (iv) 'the matrix', which occupies the space between lamina densa and the collagen fibers.[32] The process of formation of the MTJ can be explained as follow: during embryonic development, nondifferentiated tendon and muscle precursor cells cohabitate at the site of future MTJ development. In response to the contractile force of the developing muscle during the late fetal and early neonatal period, tenocytes and extracellular matrix components start to align themselves in the direction of the applied force.[33] Tenocytes begin to form longitudinal rows of cells in the proximity of the MTJ. In adult tendons, neighboring rows of cells are separated by large deposits of type I collagen and other extracellular matrix components. Therefore, in adults, tendons are not simply composed of a number of individual cells dispersed in ECM, but are

a cohesive structure in which all the cells are interconnected via long cytoplasmic processes and form an extensive network for cell-to-cell communication.[34]

Current clinical techniques for BTJ and MTJ healing

Tendons can suffer two kinds of acute failure: rupture from extreme instantaneous loads and accidental lacerations. The longitudinally linear organization of collagen fibers and fascicles in tendon results in optimal stiffness and strength at low strains under tensile load; this organization makes a biologically sound repair of ruptured or lacerated tendons extremely difficult to be achieved.

To improve the clinical results, tendon transfer or autografting are routinely performed.[35] However, these techniques present some disadvantages. For example, tendon transfer is not necessarily an anatomical reconstruction, and autografts require the sacrifice of healthy and functional tissue. However, tendon grafts continue to be considered a viable option for tendon reconstruction. Experimental studies have focused on tendon allografts[36,37] and xenografts,[38] with the problems relative to the antigenicity of grafted tendons. An alternative is the use of extracellular matrices derived from animals, such as porcine small intestinal submucosa; however, tissues derived from animals may cause immune reactions and zoonoses.[39,40] Therefore, they must be accurately treated to reduce or eliminate antigenicity. Tendon healing also involves the participation of fibroblasts from the peritenon (synovial sheath), epitenon and endotenon itself.[41] The relative contribution of each cell type depends on the type of trauma, anatomical position of the repair, the presence or absence of a sheath and post-repair motion stress. The best biomechanical repair with the least complications is not necessarily the one that produces the fastest healing, which is achieved if the intrinsic endotenon fibroblasts populate the repaired site and produce the appropriate ECM.

The above techniques present several limitations. First, the growth of new tissue requires several weeks, and during the initial recovery phase, the healing tendon cannot be fully loaded. In most instances, partial or full immobilization is required during the period of repaired tissue growth. Second, the repaired tissue lacks appropriate mechanical strength, because the void is filled by a disorganized, matrix-rich, scar-like material.[42]

BTJ and MTJ healing suffers the same problems. From the first studies,[43] it appeared that the level of bone ingrowth was the most important factor that influenced the mechanical strength of interfacial healing. From a clinical point of view, using ACL reconstruction as a model, the hamstring tendons graft is mechanically fixed within the femoral and tibial bone tunnels. While these grafts may restore the physiological range of motion and joint function through mechanical fixation, biological fixation is not achieved because disorganized scar tissue forms within the bone tunnels.[44] Without a functional interface, the graft-bone junction exhibits limited mechanical stability, and the lack of graft integration constitutes a primary cause of graft failure.[45,46]

In muscle injury, the sarcolemma of myofibers is usually damaged, resulting in various degrees of necrosis, which often disrupts the functional continuity of the tendon–myofiber–tendon units.[47] Regeneration of the injured myofibers and nerves and formation of connective scar tissue between the stumps are two simultaneous processes that are at the same time supportive of but also in competition with each other. On the other hand, if connective scar tissue formation between the stumps is excessive, it may prevent the regeneration of myofibers and re-innervation of the stumps.[48] During early regeneration, myoblasts and myotubes can merge with the surviving parts of the transected myofibers. However, it is not known whether it is possible that opposite stumps could eventually merge to assemble the divided parts of the transected fibers.[49]

Proposed tissue engineering applications for BTJ and MTJ

Biological scaffolds

Biological scaffolds are prevalently obtained from porcine or bovine mammalian tissues.[16] These materials can ideally maintain the native structure and composition of the tissue ECM from which they

derive. As they are xenogenic, when implanted they could promote a proinflammatory reaction. However, when properly processed to effectively remove resident cells and produce minimal distortion in the native micro and nanostructure, they could induce a macrophagic response which can properly guide the proinflammatory reaction.[50] Nevertheless, when in biological scaffolds (cross-linked or not) resident cells have not been completely eliminated, fibrosis or scar tissue can be formed.[51–53] To remove any non-collagen component, small intestine submucosa (SIS), dermis and pericardium are usually processed through several steps, including general cleaning, removal of lipids or fat deposits, disruption of cellular and other DNA materials, cross-linking and sterilization.[50] The final scaffolds are mainly composed of naturally occurring collagen fibers, predominantly type I collagen, and several of them have a bioactive surface chemistry and native structure that is bioactive and can promote cellular proliferation and tissue ingrowth.[50]

Therefore, the use of biological scaffolds produced from human or animal tissue is relatively simple in principle. However, the use of allo- or xeno-scaffolds carries the risk of disease transmission and immunological rejection if the cells and non-ECM materials are not completely eliminated.[19]

A more effective technique to eliminate all non-collagen components could be the application of supercritical CO_2 (SC-CO_2) or SC-CO_2-based mixtures that can access all nanometric spaces inside the biological structure, given their gas-like properties, and can then efficiently eliminate undesired organic matter.[54–56]

Some biological scaffolds are commercially available[57] and are used to test musculotendinous repair in addition to stem or progenitor cells;[58–67] however, the MTJ is specifically considered only in rare cases. For example, Turner *et al.*[68] studied the capacity of an ECM-based scaffold to facilitate functional restoration of the distal gastrocnemius MTJ in a canine model after complete resection of the tissue. Within 6 months, a vascularized and innervated skeletal muscle, similar to normal muscle tissue, was formed at the ECM-scaffold implantation site. This work represents the first report of *de novo* formation of contractile, vascularized and innervated skeletal muscle *in situ*. Valentin *et al.*[69] investigated both the *in situ* tetanic contractile response and the histomorphologic characteristics of skeletal muscle tissue reconstructed using four different materials in a rodent abdominal wall model: (i) porcine SIS-ECM; (ii) carbodiimide-cross-linked porcine SIS-ECM; (iii) autologous tissue; or (iv) polypropylene mesh. The implant materials were chosen since they represented the spectrum of possible surgical solutions to soft tissue or musculoskeletal repair. Six months after surgery, the remodeled SIS-ECM showed almost complete replacement by islands and sheets of skeletal muscle, which generated a similar maximal contractile force to native tissue, but with greater endurance. The autologous tissue graft was replaced by a mixture of collagenous connective tissue, adipose tissue with fewer islands of skeletal muscle compared with SIS-ECM and a fatigue endurance similar to native muscle. Carbodiimide-cross-linked SIS-ECM and polypropylene mesh were characterized by a chronic inflammatory response and produced slight or not measurable tetanic force.

The use of acellular scaffolds overcomes many of the hurdles associated with cell seeding, such as the necessity of prolonged *in vitro* cell culture, or the requirement for specific bioreactors that provide physiological stimulation to the cells for promoting differentiation;[57] however, the complete elimination of non-ECM materials remains one of the key problems of this technique.

Administration of active compounds to promote anchoring of the tendon

Some studies reported the administration of cytokines,[45] wrapping of periostium,[70] use of hydroxyapatite and Ca-P25[71] to promote anchoring of the tendon to bone, obtaining at least partial bone–tendon integration. In particular, cytokines play a role in tissue formation and support the potential for growth factors to improve tendon to bone healing. Nevertheless, one of the principal challenges to the use of cytokines and growth factors in tissue engineering is the identification of optimal delivery vehicles that localize the factor to the repair site for the relevant period of time and appropriate concentration.[72]

Rodeo *et al.*[45] examined the hypothesis that recombinant human bone morphogenetic protein-2 could enhance bone ingrowth into a tendon graft placed into a bone tunnel in the proximal tibia. Histological and radiographic examination showed more extensive bone formation around the tendon with closer apposition of new bone to the tendon in the case of protein-treated limb than in the paired control limb. Biomechanical testing demonstrated higher tendon pull-out strength on the protein-treated side, with a statistically significant difference between the low-dose-treated side and the control side at 2 weeks. Therefore, bone morphogenetic protein seems to accelerate the healing process when a tendon graft is transplanted into a bone tunnel.

However, the bone tunnel model does not take into account, for example, that torsion forces on the healing tendon may well promote a reaction at the interface between the tendon and bone, and histological and biomechanical properties could be influenced by these forces. Kyung *et al.* examined how the periosteum improved the healing process of a tendon in a bone tunnel.[70] Periosteum is able to generate all types of connective tissue and has osteogenic capacity. In this study, histological examination showed more extensive bone formation around the tendon, with closer apposition of new bone to the tendon in the periosteum-wrapped limb than in the control limb. Chen *et al.*[73] also tested the effect of periosteum on the healing of a tendon graft in a bone tunnel of a rabbit model. They obtained a progressive increase in tendon pull-out strength that followed bone ingrowth, mineralization and incorporation of the healing tissue. Histologic analysis of the tendon-bone interface showed a fibrous layer formed between the tendon and the bone by the periosteum; this layer became progressively integrated with the tendon and bone surface during the healing process. This technique is relatively simple and inexpensive, and can be applied in clinical practice. It does increase the operating times however, and, as it makes the graft more bulky, it requires larger tunnels, which may compromise the mechanical properties of the bones where implantation takes place.

The administration of active compounds to promote the anchoring of the interface is based on the hypothesis that it is possible to induce local restructuration of the specific tissue in the highly organized manner of the original junction. This approach is partly successful, since its application may require complex procedures and relatively long times to produce interface reorganization. It should be kept in mind that the use of periosteum may well be a low-cost, highly effective alternative to the use of sophisticated active compounds.

Tissue regeneration by natural and/or synthetic polymers

The materials normally proposed for TTE can be divided in natural and synthetic polymers.[19,20,74] The same materials could be used to reproduce MTJ and BTJ.

The ideal scaffold for interface tissue engineering must direct heterotypic and homotypic cellular interactions, while promoting the formation and the maintenance of controlled matrix heterogeneity. Consequently, the scaffold should exhibit a gradient of morphological and material properties mimicking those of the native insertion site.[75]

In general, natural and synthetic scaffolds pose no risk of disease transmission.[50] One of the advantages of using biomaterials is that exogenous growth factors, gene therapy approaches or cell delivery can be also used, but the production of structures that mimic even at nanometric level the original biological materials is still problematic.

A strategy commonly employed in tissue engineering is to combine an appropriate cell type with a suitable biodegradable scaffold to produce functional tissues *in vivo*. This strategy has been successful in developing single tissue types.[76–78] However, there is an increasing demand for methods that could allow the formation of more complex composite tissues with a coordinated function.[79]

The healing of the BTJ insertion in adults is generally characterized by the formation of disorganized tissue and lack of a fibrocartilaginous transition between tendon and bone.[80,81] Indeed, the interfaces bridge the gap between two dissimilar tissues, usually with physical and biological properties that separate them from the tissues they connect. Interfaces in orthopedics are crucial, as they transfer load

between bone, cartilage, tendon, ligament and muscle tissue.[82] Restoration of full function after the application of tissue engineering strategies is required. Nevertheless, insertions of tendon or ligament into bone vary considerably depending on the particular attachment. From a histological point of view, indirect and direct insertions are also known as fibrous or fibrocartilaginous entheses, respectively.[83]

Work in this area can be divided according to its aim: either to build a whole composite tissue unit (e.g. formation of a tissue-engineered bone/muscle–tendon graft) or to assist the regeneration of an individual interface, such as improving integration of autografts with surrounding tissue. Most current work on interface engineering has focused on assisting graft anchorage. For example, Spalazzi *et al.*[44] designed and evaluated a multiphasic scaffold with the potential to direct the regeneration of the multi-tissue interface between tendon grafts and bone *in vitro*. However, at present, it is difficult to locate materials and suture strategies that allow smooth implantation and avoid the production of stress raisers.

The most used technique to engineer BTJ and MTJ structures is electrospinning. It has been previously used as a fabrication method to develop several tissues,[84–86] and only few investigations have focused on soft tissues-related materials.[87] Using this technique, fiber composition and diameter can be modified varying the polymer, its concentration in the liquid solution and other process parameters. Nanofibers can be prepared as nonwoven mats by electrospinning from a wide variety of biocompatible and biodegradable polymers (both natural and synthetic), as well as composites containing inorganic materials.[88] A nonwoven mat derived from electrospun nanofibers typically exhibits a high porosity and large surface area because of its small size; for this reason, it could thus mimic the hierarchical structure of ECM, which is very relevant for cells attachment and nutrient transport.[89] The fibers can also be conveniently functionalized by encapsulation or attachment of bioactive species such as ECM proteins, enzymes, nucleic acids and growth factors to control the differentiation and proliferation of seeded cells. Additionally, the nanofibers can be readily assembled into a range of arrays or hierarchically structured films by manipulating their alignment, stacking and/or folding.[90]

Riboldi *et al.*[91] investigated the suitability, as scaffold for skeletal muscle tissue engineering, of a biodegradable block copolymer (DegraPol) processed by electrospinning in the form of microfibrous membranes. A promising cellular response was found in preliminary experiments: both line cells and stem cells adhered, proliferated and merged on differently coated electrospun membranes. Larkin *et al.*[3] engineered self-organized 3-D tendon through co-culture of tendon and muscle cells. The resulting scaffold-free tissue was composed of well-aligned, small-diameter collagen fibrils, a large number of cells and an excess of noncollagenous ECM. They also showed that, at the MTJ of these engineered constructs, there was an increase in the expression and localization of some of the MTJ-specific proteins, such as paxillin, similar to those found in fetal and neonatal MTJs *in vivo*. Ladd *et al.*[15] developed a scaffold characterized by both a compliant/high strain region, a stiff/low strain region and an intermediate region that would overcome the challenge of providing two different mechanical profiles, mimicking the trends observed in native tissue. The scaffold presented in this study is not without limitations: its mechanical properties did not match native MTJ mechanical properties, even though the trends in properties between scaffold and native MTJ were similar; the whole scaffold had a higher stiffness and ultimate tensile strength (UTS) than native MTJ, but a lower strain at failure. Because the overall properties of the native tissue were primarily driven by the strength and stiffness of the muscle, it is not surprising that the native tissue would exhibit a lower stiffness and higher strain at failure than a synthetic polymer scaffold. Moreover, cyclic testing revealed that the scaffolds in their current form may not be ideal, as creep occurred after relatively few cycles, compared with the number of cycles of an MTJ construct.

Summarizing, electrospinning was applied to BTJ and MTJ regeneration; the use of nanofibers that mimic the hierarchical structure of the ECM, functionalized by encapsulation of bioactive components, allowed cell attachment and differentiation. Nevertheless, electrospinned constructs are discontinuous, i.e. arrays of separated fibers are produced, with the

ensuing issue of mechanical performance integrity of the scaffolds produced.

In some, limited cases, gel drying and hydrogel freeze drying were proposed for tendon-related tissue engineering using collagen and other polymeric materials that form a relevant part of the native ECM of many tissues. Gel drying can be potentially successful also in BTJ and MTJ regeneration when mineralization gradients are imposed along the structure. Differently from electrospinning, it starts from homogeneous (single block) constructs that contain the nanostructural fiber organization typical of gels and hydrogels. Gels can be organized in any shape from the macroscopic point of view. The elimination of the organic solvents or of water from the gels avoiding the collapse of their delicate nanostructure is the most critical part of this technique.[92] Moreover, hydrogels require cross-linking to produce stable (not hydrosoluble structures), and part of their properties could be compromised. Cross-linking agents, such as glutaraldehyde, are cytotoxic;[93,94] therefore, their accurate elimination is also required.[95]

Gel drying assisted by supercritical fluids for the production of scaffolds has been recently proposed.[55,96–98] SC-CO_2 was used to eliminate the organic solvents in a simple and effective step. Reverchon *et al.*[98] tested this process for the formation of PLLA[99] and hydrogel scaffolds.[54] This technique can potentially solve the problems related to solvents elimination either during gel drying or after cross-linking.

Therefore, promising alternatives to the electrospinning technique are gel drying and hydrogel freeze drying. These techniques can be potentially successful in BTJ and MTJ regeneration when different degrees of mineralization are imposed along the structure. The elimination of the solvents and/or of cross-linking agents, avoiding the collapse of gels delicate nanostructure, is the most critical part of this technique, which can be successfully achieved by SC-CO_2-assisted processing.

Self-reorganized constructs

The use of mesenchymal stem cells (MSCs) to enhance allografts osteointegration offers the potential of more physiological and earlier healing.[100] MSCs derived from synovium have a higher proliferation and differentiation potential than other MSCs.[101] Wang *et al.*[102] designed and optimized a biomimetic co-culture model. Co-culture of fibroblasts and osteoblasts led to changes in their respective phenotypes, as well as the expression of interface-relevant markers. This study confirmed the role of osteoblast–fibroblast interactions in fibrocartilage formation and demonstrated the utility of *in vitro* co-culture models to investigate the mechanism governing the formation of the tissue-to-bone interface. The first experiments using MSCs to regenerate musculoskeletal soft tissues *in vivo* utilized autologous MSCs suspended in a type I collagen matrix.[103] Pittenger *et al.*[104] subjected human MSC/collagen gel constructs to cycles of tension to model the effects of tensile forces on *in vitro* construct maturation. The cycled tissue showed a collagen fiber organization parallel to the long axis of the tensile loading with an alignment of crimp patterns that was not evident in the static constructs. In addition, the MSCs produced increased amounts of collagen type VI, which is associated with repair of connective tissues and early maturation of normal tendon tissue.[105,106]

The attempt to induce tissues self-reorganization at a cellular level is very audacious, but, until now, the potential of this technique has barely been explored. Other studies are necessary to verify the effectiveness of this strategy.

Table 1 Techniques proposed to BTJ and MTJ regeneration

Technique	BTJ	MTJ
Biological scaffolds		✓
Administration of active compounds	✓	
Electrospinning	✓	✓
Self-reorganized constructs	✓	✓

Conclusions and perspectives

The various research strategies for BTJ and MTJ, described in this review and summarized in Table 1, are the result of the different ways of thinking about bone–tendon and muscle–tendon interfaces restoration and, more generally speaking, tissue regeneration.

The most popular techniques proposed for tissue regeneration are based on the preparation of scaffolds seeded with different types of cells and growth factors that should support tissue regeneration. At present, these studies, though promising, should be considered preliminary feasibility investigations. We have proof of concept that MTJ and BTJ can be, at least partially, tissue engineered. However, there have been, to our knowledge, no translational investigations, and we are still far from being able to apply these implants in clinical practice. Undoubtedly, novel research methods will come to the forefront and will need to be tested out in an appropriate scientific fashion. To some extent, it will be a question of Darwinian selection: methodologies that are scientifically sound and have evidence of success *in vitro* and animal studies will have to be translated into human medicine, to ascertain which are viable and confer clinical success in the management of these injuries.

Over the course of the next several years, appropriately planned studies will need to transport tissue engineering from a scientific challenge to a clinically applicable technique. This study is in compliance with ethical standards.

Conflict of interest statement

None declared.

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