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Tissue Engineering of Tendons

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INTRODUCTION

Rupture of tendons due to either high load or laceration is a common injury, especially for the hand. Tendon disorders are traditionally treated by reducing inflammation, restoring flexibility, and, if necessary, performing surgical repair.[1] It has been estimated that hand surgeons repair nearly one-third of a million digital flexor tendon injuries per year in the United States.[2] Despite the fact that surgery of tendons is a common procedure, its outcome is often unsatisfactory. Recovery after tendon surgery can take months. At best, the restored tendon is about half of its initial mechanical properties, so surgeons often face patients' dissatisfaction. On the other hand, if left untreated, healing tendon is not as good as original structure from a mechanical point of view. Moreover, the related pain is difficult to treat.

Several factors may explain these unsatisfactory results such as the difficulty to anchorage the tendon in muscle, the high mechanical constraint on the repair structure, or the unavailability of ideal tendon grafts (currently, tendon grafts are either auto, allo, or synthetic). It has been shown that early mobilization following tendon surgery gives superior results.[3] The repaired tendon should then be able to support immediate loading. However, suturing techniques to joint split ends of tendons, while providing sufficient mechanical strength to limit gapping, are inadequate to carry normal loads.

Based on the shortcomings of the actual tendon surgery, tendon tissue engineering may offer an alternative approach. The objective in developing an engineered tendon will then be the reconstruction of the injured structure with structural and functional properties as good as the original. Moreover, an engineered tendon could reduce the rate of secondary problems by making the surgical procedure less extensive.

This article gives first some general details on anatomy and physiological properties of tendons, followed by a list of requirements for an ideal engineered tendon. A review of the actual state of the art in tendon replacement is then performed, and finally, some remarks on the future of tissue engineering of tendons are proposed.

ANATOMICAL AND PHYSIOLOGICAL PROPERTIES OF TENDONS

Tendons are dense, regularly organized, and by their arranged collagen fibers, allow connection between muscles and bones. The structure is composed of long arranged collagen fibers, and the function and behavior of tendons depend on of their mechanical properties.

They are capable of resisting important strength in order to transmit the muscular power to the bones and articulations. They protect the muscle (fascia) and fix the length of the muscle belly. They also play a role of damper and shock absorber.

A tendon is composed of multiple fascicles surrounded by a fascicular membrane and septa. Inside the fascicle, we find a mix of fibroblasts in relative central position and fibrils. The fibrils are composed of microfibrils, which themselves are made of tropocollagen.

The fascicular membrane is the endotenon, which is draped over by a sliding structure, the peritendon. Between the peritendon and the endotenon run vessels, nerve, and lymphatics. Depending on the localization, the organization of the surrounding layers is more complex.

A hand flexor tendon is encircled by a well-defined sheath covered by synovial cells. In this situation, the external layer (paratenon) is called tenosynovium, but if there is no synovial lining, the paratenon is called tenovagium.

At the musculo-tendinous junction, the perimysium is continuous with the endotenon. At the other extremity on the tendon–bone interface, the collagen fibers and the endotenon become continuous with the periosteum.

The tendon insertion into bone is of two types. The first one described by Cooper and Misol,[4] shows a transition from the tendon to a layer of fibrocartilage with digitations in the periosteum. The fibrocartilage is

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progressively ossified and merges with bone. The second one is more complex and is characterized by having the periorteum as an intermediate layer. Superficial fibrils are fixed into the periosteum and deeper, directly into bone.

Tendon is largely vascularized, the blood flow coming from the perymisium, periosteum attachment, and surrounding tissues. In particular, the perfusion through the surrounding tissue reaches the tendon penetrating the paratenon, the mesotenon, or vincula. We can separate vascularized from avascular tendons: vascularized tendons concern those with a rich surrounding vascularization, which penetrate directly inside the endotenon. The avascular tendons are contained in a sheath, and the vessels are penetrating through thin structure (mesotendon or vincula), which reticulate and bind the tendon.

The innervation plays a sensory role through mecanoreceptors located near the musculo-tendinous junction.

REQUIREMENTS FOR TISSUE ENGINEERING OF TENDON

Several requirements need to be fulfilled in the tissue engineering of tendons. Some are common to most engineered tissues used for applications in musculoskeletal applications, but some are specific for the tissue engineering of tendons.

Common Requirements in Tissue Engineering

1. It is usually stated that an engineered tissue is composed of three elements: scaffold, cells, and growth factors. Tissue engineering is then, by definition, multi-disciplinary. In order to bring useful and adequate solutions, it is especially important that physicians, who will be the users of the developed engineered tissue, are involved at every step of the project development. For example, surgical procedures should be anticipated since the beginning of the project to insure that the developed tissue will be manageable in the constraint environment of a surgical room.
2. As a basic rule, the engineered tissue should be easy to use and its manipulation should be as close as possible to the actual standard surgical procedures.
3. The engineered tissue will need to be approved by governmental agencies (e.g., FDA) before being available on the market. Especially if cells and/or growth factors are used, it is important to be aware of the tests required by these governmental agencies to obtain an approval for clinical use. This would avoid the repetition of experiments not following established standards.

Specific Requirements for Tendon Tissue Engineering

1. Tendon auto-grafts are freely available during surgery. Due to economical constraints on the health care system, it would be difficult to propose an expensive engineered tendon. The only economical factors, which may favor the use of an engineered tendon from the health insurance point of view, would be the reduction of the operative time and a faster patient recovery. Based on these economical considerations, the production and stock management of the engineered tendons should be considered from the beginning of the project.
2. Tendon biomechanics is complex due to its nonlinear stress–strain relationship and viscoelasticity (Fig. 1).[5,6] In-vivo forces quantification of tendons is also a useful approach for the determination of

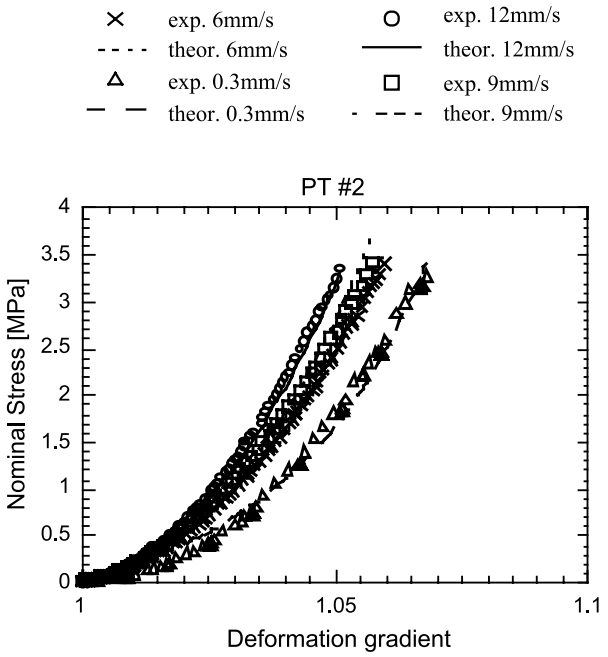

Fig. 1 Experimental and theoretical stress–strain curves obtained at four different rates of elongation for one human patellar tendon specimen. Good correlation between experimental and theoretical curves are found. The proposed constitutive law with only three parameters is then able to fit nonlinear stress–strain curves obtained at different strain rates. (Reproduced with permission from Ref. [6].)

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mechanical design parameters.[7] An engineered tendon should be able to reproduce these mechanical behaviors. It should be noted that different tendons might present different mechanical properties as has been shown for fatigue damage.[8]

3. Fixation on bone and muscle may be the weakness mechanical part of the engineered tendon. It is then important to incorporate this point in the design of the tendon. For example, it has to be decided early in the project if the scaffold will be sutured or glued to the muscle. In case of high mechanical constraints (early loading), it would be more advisable to suture than to glue the tendon. However, the drawback of tendon suturing is the possibility of inducing a devascularization of the tendon and a corresponding necrosis of the tissue. It should be noted that if left untreated, some tendons might spontaneously heal, suggesting that the gluing process of tendon to muscle is a normal way of healing.
4. Tendon cells must communicate to sustain growth and matrix expression.[9] It has been shown that cells in the epitenon and internal compartment of whole tendon are connected physically to each other and express gap junctions.[10] Successful engineered tendon replacements must include designs that allow for cell-to-cell connectivity to allow intercellular communication. Scaffolds should then be easily seeded by cells and encourage cell division and matrix expression.[9]
5. If tendon fibroblasts are used, it has to be noted that these cells are site-dependent and may induce different results regarding proliferation and/or gene expression.[11,12] Autologous cells' transplantation has the advantage of not inducing immune reaction and is more easily implemented from a regulatory point of view. However, an initial biopsy followed by a cellular expansion period is necessary, rendering this approach time consuming. Disadvantages of autologous cells' transplantation disappear with allogous cells' transplantation, but the benefit of no immune reaction and easier regulatory implementation are, on the other hand, lost. A careful selection of the cells used, depending on the application, should then be performed.

ACTUAL APPROACHES FOR TISSUE ENGINEERING OF TENDON

In the term tissue engineering, we consider all the engineering and biological approaches used in the development of an artificial tendon. Different solutions proposed for treating tendon rupture are presented from the scaffold, cells, and growth factor/gene therapy points of view.

Scaffold

In order to bridge tendon lesions, synthetic scaffolds have been used with relatively modest success.[13] Synthetic tendon made of Dacron™, nylon, or even carbon fibers has been used. One of the only synthetic scaffolds remaining on the market is the Leeds-Keio ligament made of polyethylene terephthalate (PET). Recently, it has been shown that treatment with radiofrequency (RF)-generated glow discharge increased cell attachment and proliferation on this PET implant.[14]

Organic polymers' matrix, such as acellular collagen, have also been employed as tendon graft.[15] These scaffolds are supposed to provide early mechanical support and promote cellular infiltration. However, achilles tendon repairs using collagen scaffold achieved only 36% of normal maximum force.[16] Naturally occurring extracellular matrices were also proposed for tendon repair. There are limited locations within the body that can be used as the source of these extracellular matrices. One is derived from porcine small intestinal submucosa.[17] With this tissue used as tendon scaffold, a transient and significant weakening of mechanical properties was found compared to patellar tendon in the first months after implantation in an animal study. After 12 months, no difference was found. In order to increase the mechanical properties of the scaffold, collagen scaffold with poly-L-lactic acid was developed.[18] Mechanical properties were increased with this method. Probably one of the most promising scaffold developments from biomechanical and biocompatible points of view would be based on biomimetic strategy. In this approach, the scaffold should include polymeric collagen as the fundamental fibrous phase and being cross-linked to give the mechanical strength of the engineered tendon. To this end, derived from skate egg capsule, a collagen-based scaffold was developed.[11] Fibers produced from pepsin-solubilized, bovine tendon type I collagen were polymerized with di-catechol nordihydroguaiaretic acid (NDGA). These NDGA cross-linked fibers showed high mechanical properties comparable to native tendon and demonstrated excellent biocompatibility properties.[19]

Cells

As for other engineered tissues (e.g., bone, cartilage), incorporation of cells is supposed to increase the healing potential of the tissues. A combination of scaffold and cells is then developed, the scaffold playing either the role of cell delivery system alone or cell delivery system and mechanical support.

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Use of autologous mesenchymal stem cells seeded in collagen gel in vitro and then implanted in the body has been proposed.[20] The results indicated that delivering mesenchymal stem cell–contracted, organized collagen implants to large tendon defects could significantly improve the biomechanics, structure, and probably the function of the tendon after injury.[21] Mesenchymal stem cells, which have the potential to differentiate in (tendon) fibroblasts, hold the promise to be used for allogous cells' transplantation. However, if the cells are not oriented in the gel, only modest improved biomechanical properties are observed compared to natural repair of unfilled defect.[22] The orientation of the cells in the gel, in particular due to the gel contraction mediated by cells, improved the biomechanical propreties.[23] Besides mesenchymal stem cells, it may be useful to use differentiated fibroblasts in order to speed up the healing process. Moreover, fibroblasts seeded on a scaffold and in vitro mechanically stimulated the mechanical properties of the graft.[24] So, it has then been proposed that the delay between rupture and surgery may be used to engineer the tendon in vitro by applying mechanical stimulus. This approach may, however, be difficult to apply from an economical point of view. In order to amplify the potential of cells in the healing process, autologous mesenchymal stem cells might be genetically modified to produce growth factors useful in the healing process of tendon.[25]

Growth Factor/Gene Therapy

Use of growth factors is related to a good understanding of the tendon healing process. This healing process could be viewed as four overlapping phases: hemorrhagic, inflammatory, proliferation, and remodeling/maturation. The second phase, inflammatory, is probably a key phase as many different growth factors and cytokines are produced and will drive the outcome process of healing. A detailed description of these phases may be found in Ref. [26]. It has been shown that treatment with growth factors such as PDGF and IGF-1 stimulate DNA synthesis of tendon fibroblasts.[27] However, as for other tissues, issues such as the difficulty to deliver the growth factors or the supraphysiologic concentrations needed to induce an effect[28] need to be solved. A potential solution might be the use of gene therapy. Genetically modified cells[25,27] or gene-activated matrix[29] might be used to target specific location (ruptured zone of the tendon) and specific biological process (healing of the tendon). This approach is still in its infancy, but promising results have already been obtained.

FUTURE DEVELOPMENT FOR TISSUE ENGINEERING OF TENDONS

As mentioned by Hildebrand,[25] the regeneration of normal tendon has not been achieved and further refinements are necessary for these treatments. Indeed, none of the actual solutions followed all the requirements noted earlier for tissue engineering of tendons. Especially, problems like fixations of tendons, incorporation of biomechanical properties, choice of scaffold with adequate degradation properties, or packaging of the engineered tendon[20] still need to be solved. Nature used millions of evolution years to optimize the functionality of the tendons. It might then be worthy to use this knowledge in order to engineer tendons. The combination of biomimetism, as proposed by Koob[11] to engineer tendon scaffold, and gene therapy, either with cells, e.g., Ref. [25], or with gene activated matrix[29] to target specific healing tendon process, held probably the most promising development in tissue engineering of tendon.

CONCLUSION

Important progresses have been achieved recently in tissue engineering. Although no ideal solution exists for tissue engineering of tendons, it is reasonable to believe that good solutions will emerge from laboratories. In particular, the combination of biomimetism and gene therapy with mesenchymal stem cells opens new fields of possibilities from a scientific and clinical point of view. However, the development of tissue engineering of tendons can only be successful if the challenging economical constraints are satisfied. Based on the past industrial experiences in tissue engineering for bone or cartilage, it would be an illusion to believe that engineered tendon with cells may be entirely supported by private industries. It seems more reasonable to develop collaborations between industries and hospitals. The industries would be responsible for developing and managing the scaffolds, and the hospitals would be responsible for managing the cells' bank and seeding process of the scaffolds with the selected cells. With this kind of strategy, it is hoped that, in the future, surgeons will no more face the paradox of treating a ruptured tendon by collecting another tendon in the patient.

ARTICLES OF FURTHER INTEREST

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