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Prospects of Tendon Tissue Engineering in Sports Medicine

Aussichten für das Sehnen-Tissue Engineering in der Sportmedizin

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ZUSAMMENFASSUNG

Die Prävalenz von Sehnenverletzungen steigt teilweise durch die vermehrte Ausübung von Freizeit- und Leistungssport. Primäre chirurgische Behandlung wird immer noch als Goldstandard in der Behandlung solcher Verletzungen angesehen. Jedoch stellen Transplantationen in Situationen in denen Gewebeverlust vorkommt immer noch eine klinische Herausforderung dar. Während historisch betrachtet viele Transplante aus Biomaterial zur Sehnenrekonstruktion benutzt wurden, haben Fortschritte im Tissue Engineering und in der Herstellung von faserigen Gewebsgerüsten hochentwickelte Transplantationsmöglichkeiten der klinischen Umsetzbarkeit näher gebracht. Stammzellen und Biomaterial spielen eine große Rolle im Tissue Engineering. Sie liefern biologische Einheiten bzw. Stützgerüste und unterstützen somit die Wiederherstellung der richtigen Sehnenstruktur. Diese werden von Wachstumsfaktoren und Signalen begleitet, die die Wundheilung oder die Entwicklung des Gewebes während der Embryogenese regulieren. In diesem Beitrag wurde der biomimetische Ansatz einer Sehnentransplantation untersucht, der die mesenchymale Herkunft der Sehnenurform, die Faktoren, die die Differenzierung dieser Zellen innerhalb der biologischen Entwicklung der Sehne beeinflussen und die Herstellungstechnologien der Zellträger, die das Potenzial haben, die Struktur der Fasermatrix im Design des biomateriellen Trägers zu replizieren, berücksichtigt. Der Beitrag zeigt die aktuellen Konzepte, Fortschritte sowie entstehende Erkenntnisse von Paradigmenwechsel und technologischen Durchbrüchen, die das Thema zukünftiger Forschung sein werden.

Schlüsselwörter: Sehne, Mesenchymal Stammzellen, Wachstumsfaktoren, biomaterielle Zellträger, Tissue Engineering, erneubare Medizin.

SUMMARY

The prevalence of tendon injury has been rising due, in part, to increased participation in recreational and competitive sports. Primary surgical repair still represents the gold standard in the treatment of these injuries. However, graft transplantation in scenarios that involve tissue loss still presents clinical challenges. While many biomaterial grafts have historically been used in tendon reconstruction and most have since fallen out of favor, advances in tissue engineering and fibrous scaffold fabrication have made the use of engineered grafts closer than ever to clinical translatability. Stem cells and biomaterials play a prominent role in tissue engineering as they provide the biological units and the scaffolding, respectively, to support the regeneration of the proper tendon structure, which is also guided by growth factors and signals that regulate wound healing or the development of the tissue during embryogenesis. In this review, we examine a biomimetic approach to engineer a tendon replacement that takes into account the mesenchymal origins of the tendon progenitors, factors that influence the differentiation of these cells within the framework of developmental biology of tendon, and scaffold fabrication technologies that have the potential to replicate the structure of the fibrous matrix in the design of the biomaterial scaffold. In doing so, the review presents current concepts and advances, and emerging evidence of paradigm shifts and technological breakthrough that will predictably be the subject of future research.

Key Words: Tendon, mesenchymal stem cells, growth factors, biomaterial scaffolds, tissue engineering, regenerative medicine.

INTRODUCTION

In the United States alone, nearly 61 % (61.2 million out of 100.4 million annually) of all injury treatments in physician offices, emergency rooms, outpatient clinics, and hospitals are due to musculoskeletal injuries. Soft tissue injuries including sprains, strains, and ruptures of tendons and ligaments account for 30 % of these injuries, exceeding the incidence of bone fractures , and costing the health care system in excess of $30 billion a year (34). While the prevalence of such injuries in the workplace has progressively declined over the past two decades due to the implementation of occupational safety policies (20), sports-related injuries have been rising due to increased participation of the casual athlete in recreational sports, and the increasing intensity of these activities. Despite the rarity of spontaneous major tendon ruptures (MTRs), the prevalence of injuries involving major structures such as the quadriceps tendon (QT), patellar tendon (PT), and Achilles tendon (AT) has increased in recent decades (50). For example, the rate of AT ruptures, which are common in active men in the fourth or fifth decades of life, increased from 2 to 12 in 100.000 in the past decade, in association with strenuous sports activities (19,25).

Rotator cuff injuries are also among the most common of sports-related trauma, accounting for nearly 4.4 million physician visits annually. Participants in overhead sports are the most susceptible to rotator cuff injuries, and often experience partial and complete rotator cuff tears (51). These injuries frequently require a challenging re-attachment of the tendon to the bone, and the successful outcome hinges upon recreating the gradual transition from soft tissue to mineralized tissue through a fibrocartilaginous transition zone (42).

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These soft tissue injuries can dramatically affect a patient's quality of life, and as the aging population becomes increasingly active the frequency of these injuries is expected to persistently rise. For example, the likelihood of rotator cuff tear increases from 13% in the population between the ages of 50 and 59 to over 51% in the aging population over the age of 80 (7). Failure to establish normal tissue strength in these repair scenarios can lead to recurrence of injury and in cases associated with infections the outcome can be devastating (31).

In cases where tissue loss is unavoidable as in massive trauma, infection, or neglected primary surgery, the use of tissue grafts in reconstructive surgery is commonplace. Despite clinical preference to using autografts, several studies have demonstrated that allografts may provide an effective functional alternative to live autografts (13,33,49). However, this is hindered by donor shortage and immunological problems associated with infectious diseases and graft-host mismatch. Therefore, there is a compelling need to pursue innovative strategies like tissue engineering and regenerative medicine to create tissue "spare parts".

STRUCTURE AND BIOLOGY OF TENDON: INSIGHTS FOR TENDON TISSUE ENGINEERING

In tissue engineering, combinations of cells and bioactive molecules are seeded onto three-dimensional biomaterial scaffolds (16,22,37,40,41,46,47,53). To date, growing functional engineered tissues in vitro for subsequent implantation into tissue defects in vivo remains experimental, although early clinical successes are worth noting (24). Despite extensive research, regenerative repair of tendon using tissue engineering still faces many challenges and fails to recapitulate the exquisite structure-function relationships in this mechanical connective tissue.

Tendon is a highly organized tissue made primarily of fascicles of fibrous type I collagen. Microscopically, collagen fibrils (100-200 nm), held together by inter- and intra-fibrillar cross-links, are assembled into fibers (50-100 µm). These fibers are then bundled in a glue of a hydrated proteoglycan rich matrix into the fascicles of the tendon. This fascinating hierarchical organization ensures that the biomechanical properties of composite tissue are greater than the sum of its microscopic components, and contributes to the tissue's viscoelastic behavior. In addition, collagen fibers exhibit a characteristic crimp pattern that contributes to tendon's nonlinear elastic behavior. Tenocytes are the primary cell of tendon, with the terms tenoblasts or fibroblasts sometimes used interchangeably in the literature. Tenocytes are elongated, spindle shaped cells that reside in between the densely packed collagen fibers. In the axial skeleton, tenocytes are developmentally derived from tendon progenitors (TNPs) in the sclerotome compartment of the somites in response to fibroblast growth factor (FGF) signaling (6), whereas transforming growth factor (TGF-β) signaling induces, maintains, and recruits TNPs in the developing tendons of the appendicular skeleton (35).

In this review, we examine a biomimetic approach to engineer a tendon replacement that takes into account the mesenchymal origins of the tendon progenitors, the factors that influence the differentiation of these cells within the framework of developmental biology of tendon, and examine scaffold fabrication technologies that have the potential to replicate the characteristic transversely isotropic fibrous matrix in the design of the biomaterial scaffold.

MESENCHYMAL STEM CELLS IN TENDON TISSUE ENGINEERING

Due to their demonstrable multipotency, marrow-derived mesenchymal stem cells (MSC) (5,32,48) and adipose-derived stromal or stem cells (ADSC) (3,17) have been the subject of extensive research for musculoskeletal tissue engineering. Recently, tendon-resident progenitors have also been identified and termed tendon stem cells (TSC) (58), and their multipotency has been demonstrated (45). And while most stem cell sources, including adipose and tendon tissue, require time-consuming enzymatic or mechanical tissue dissociation to isolate the stem cells, marrow-derived MSC have the distinct advantage of easy and quick isolation isolated using a density gradient and a centrifuge, and can be selectively enriched based on the expression of certain surface markers.

Several studies have demonstrated the efficacy of MSC in tendon or ligament repair (1,2,30,43,57). For example, Young et al (1998) reported the use of marrow-derived MSC and collagen gel constructs to repair rabbit Achilles tendon gap defects (57). This study demonstrated that the structural properties of the repaired tendon (stiffness and maximum force to failure) achieved values close to 200% of their contralateral controls (which were repaired with suture only) as early as 4 weeks post transplantation. However, the material properties (modulus and maximum stress) significantly improved overtime, with 59% to 93% increases over controls, but were only 30-40% of normal Achilles tendon properties at 12 weeks (57). Further, the incremental rate for the MSC-treated tissues was significantly greater than that for the treated controls, suggesting that while MSC transplantation in a collagen gel construct accelerated the return toward normal properties, achieving normal material properties to support in vivo loads and minimize the risk of re-injury has not been achieved. In a series of elegant studies, Butler and co-workers augmented the MSC-seeded gel with a type I collagen sponge and demonstrated further increases in repair stiffness and maximum force, such that the repair tangent stiffness matched normal stiffness (7), and recently showed that mechanically stimulating these MSC-seeded constructs in bioreactors further enhanced repair biomechanics, approaching normal properties (12,29).

Others have demonstrated that MSC can repopulate decellularized tendon allografts and differentiate into the characteristic spindle-shaped morphology of tenocyte-like cells (30,36), although the efficacy of these approaches in vivo has yet to be demonstrated in long term studies.

While the original paradigm in stem cell-based tissue engineering assumed that the primary role of MSC is to produce and remodel the repair matrix, new insights suggest that these cells release bioactive factors that make them truly pleiotropic cells with immunomodulatory, chemotactic, angiogenic, anti-apoptotic, and anti-fibrotic effects, in addition to their classical role in supporting the growth and differentiation of cells and tissues (26). This paradigm shift has important clinical and translational consequences. The pleiotropic effects of these cells might be sufficiently achieved with small numbers of freshly isolated cells that could be isolated and applied during surgery at a point-of-care without the need for costly and logistically demanding in vitro expansion and culture. However, there are no data available to determine what trophic properties of MSC are most beneficial for tendon repair.

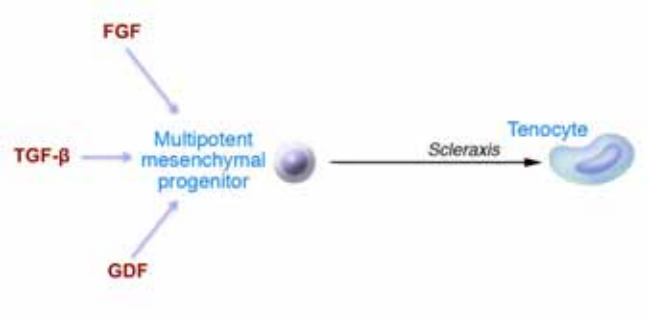

Figure 1: Factors involved in fetal development of tendon that could be therapeutic targets for tendon tissue engineering.

GROWTH FACTORS IN TENDON TISSUE ENGINEERING

A variety of growth factors, including insulin-like growth factor (IGF-1), platelet derived growth factor (PDGF-BB), TGF-β1, FGF, and GDFs, have been investigated in tendon tissue engineering (39). The rationale for investigating these growth factors is typically derived from their roles in wound healing, the expression of their genes and receptors in tendon cells in normal and healing tissue (15,56), or their role in the embryonic development of the tendon (6,35). The latter offers the most intriguing potential for regenerative tendon tissue engineering. For example, FGF signaling plays an important role in the induction of tendon progenitors (TNPs) in the axial skeleton. In the somites, FGFs emanate from the myotome to induce adjacent sclerotomal cells to become TNPs. The subsequent condensation and differentiation of TNPs is dependent on the transcriptional activity of Scleraxis (Scx) (6). On the other hand, TGF-β signaling is essential for the formation of limb tendons. TNPs positioned between the differentiating muscles and cartilage express Tgfb2 or Tgfb3 in the developing limb tendon (35). Furthermore, disruption of TGFβ signaling in $Tgfb2^{-/-};Tgfb3^{-/-}$ double mutant mouse embryos results in the loss of most tendons and ligaments in the limbs (35). Interestingly, Scx loss of function disrupts the formation of the long force-transmitting tendons and intermuscular tendons, but does not seem to affect Short-range anchoring tendons and ligaments (35).

GDFs are expressed at the interface between mesenchymal condensations in the developing synovial joints and the distal tips of digits adjacent to tendons in mouse embryos (52), where they play important roles in joint cavitation and digit separation, but their role and signaling in tendon development is not clear. This lack of clarity notwithstanding, it has been shown that subcutaneous implantation of human GDF-5, GDF-6, or GDF-7 in ectopic sites in rats leads to the formation of a connective tissue rich in type I collagen fibers that displays a crimp pattern and periodicity similar to that of tendon (52). In mice, GDF-5 deficient Achilles tendons were shown to be structurally weaker and more compliant than wild type. GDF-6 deficiency in mice leads to substantially lower levels of tail tendon collagen content, which has direct functional consequences for the mechanical integrity of the tissue. GDF-7 deficiency produces subtle effects on the composition and ultrastructure of mouse Achilles tendon (14,27,28). More interestingly, GDF-5 deficiency has been shown to delay Achilles tendon healing in mice (11), whereas GDF-5-coated suture repairs were able to stimulate a more robust healing in an Achilles tendon model in rats (38).

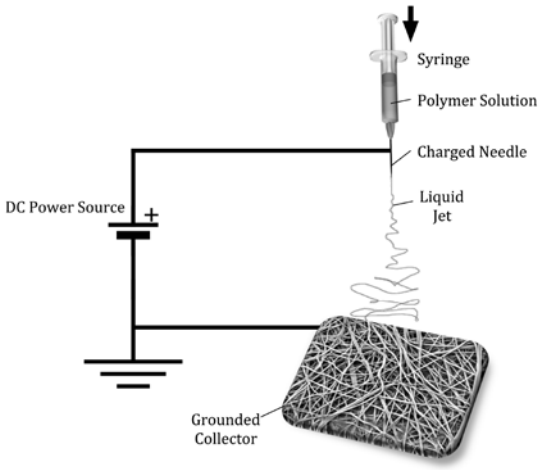

Figure 2: Engineering biomaterial scaffolds for tendon tissue engineering using the electrospinning fabrication technique.

Understanding the signaling events of these factors and how they affect mesenchymal stem cell differentiation to tendon cells (Figure 1) and how they might stimulate or repress the immunologic and vascularization responses that lead to the formation of scar tissue are not well understood and represent an area of research that is crucial for significant advances in regenerative tendon healing.

BIOMATERIAL SCAFFOLDS IN TENDON TISSUE ENGINEERING

Biomaterials play a prominent role in tissue engineering as they provide the scaffolding to support cell growth into a desired tissue or organ. Despite many advances, tissue engineers have faced significant challenges in repairing or replacing tissues that serve a predominantly biomechanical function, such as tendon. A recent report reviewed commercial biomaterial scaffolds for tendon and ligament repair focusing specifically on clinical studies (10). The report cited the major adverse events of these scaffolds, which can lead to surgical failure, and identified critical areas of improvement that "should focus on both mechanical properties and biocompatibility". The report suggested that nanofiber scaffolds fabricated using electrospinning technology might be able to offer such improvements due to the ability to control the formation of fibers on the meso/micro/nano diameter scales (10). While there are a variety of scaffold fabrication methods including drawing, template synthesis, phase separation, self-assembly, and 3D printing, electrospinning has unique advantages for scalability and the versatility to engineer the complex architecture and anisotropy in biomimetic fibrous scaffolds (8,9,21,55).

In a typical electrospinning process, a highly viscous polymer solution dispensed by a syringe at a specific feed rate forms a droplet, which is held by its surface tension at the tip of the needle (Figure 2). A voltage applied to the needle results in the formation of a Taylor cone, which overcomes the droplet surface tension and results in the eruption of a jet of the polymer solution. The liquid jet is stable near the tip of the nozzle, but undergoes instabilities including whipping and bending that lead to accelerated drying and solvent evaporation to form solid fibers, and a thinning of the fibers as the jet approaches a grounded collector (23,54).

To engineer fibrous tissues such as tendon using electrospinning, dense parallel packing of electrospun fibers is often required to achieve the native tissue's biomechanical properties. In such applications, the conventional approach of fabricating the scaffold using electrospinning and then seeding it with cells has proven to be an insurmountable challenge in terms of achieving substantial cellular infiltration within the scaffold due to the limited space between scaffold fibers (4). This results in inhomogeneous cell distribution concentrating mostly at the surfaces of the scaffold, which subsequently leads to inhomogeneous matrix synthesis and the development of a necrotic core within the scaffold. This can greatly hinder the translation of this technology to clinical applications in regenerative medicine. Increasing the porosity and spacing between the fibers of the scaffolds, via the introduction of sacrificial fibers (4), for example, to improve cell infiltration may not be an acceptable option as it generally results in reduced mechanical strength. As an alternative to conventional seeding techniques, "Bio-electrospraying" of cells onto scaffolds has recently been shown to be feasible with negligible physiological stress to the cells. A recent report has documented the feasibility of this approach in tissue engineering by electrospraying smooth muscle cells (SMCs) concurrently with electrospinning of a biodegradable polymeric small-diameter conduit scaffolds, which when cultured in bioreactor systems produced "strong and flexible tubular conduits with mechanical behaviors that mimicked those of native arteries" (44). Others have demonstrated that electrospraying of human cells induces no detectable damage at the genomic or genetic level (18). However, none of these studies have addressed the long-term viability and the biological activity of the cellular constructs in vitro or in an in vivo model of tissue regeneration. Therefore, optimizing concurrent electrospraying of cells and electrospinning of fibrous scaffolds in a one-step fabrication process might overcome the challenge of achieving optimized cellular infiltration in densely packed fibrous scaffolds and could be an exciting area of investigation in tendon tissue engineering.

CONCLUSIONS

Tendon tissue engineering has the potential to revolutionize surgical reconstruction of tendon in sports medicine, especially in cases where primary repair is not possible. For the field to move towards clinical translation, significant advances must still be achieved in our understanding of how to engineer cellular regenerative responses using factors that guide the embryonic formation of tendon. Biomimetic approaches in fibrous scaffold fabrication, such as electrospinning, hold great promise in guiding tissue regeneration to restore the anisotropic structure of the tissue, which ultimately affects its biomechanical integrity and function.

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