Orthobiologics in Achilles Tendinopathy

Subjects: Orthopedics

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Orthobiologics are biological materials that are intended for the regeneration of bone, cartilage, and soft tissues. Achilles tendinopathy (AT) or tendinitis of the heel is one of the most common ankles and foot overuse injuries. This musculoskeletal disorder usually affects professional and recreational athletes who engage in vigorous physical activities, such as jumping and running, but it may also develop in sedentary individuals. Achilles tendon injuries are often quite devastating because, unlike some tissue types, tendons are poorly vascularized structures that rely upon synovial fluid diffusion to provide nutrition. The typical features of Achilles tendinopathy are failed healing responses, persistent inflammation, and predominant catabolic reactions. Therefore, the application of orthobiologic tools represents a viable solution, considering their demonstrated efficacy, safety, and relatively easy manipulation. Perhaps a synergistic approach regarding the combination of these orthobiologics may promote more significant clinical outcomes rather than individual application.

Keywords: orthobiologics ; Achilles tendinopathy.

1. Cellular Therapy in Achilles Tendinopathy

By standard definition, orthobiologics are products derived from substances naturally found in the body that can expedite and improve the healing process of an orthopedic injury [1]. The most widely cited examples include embryonic stem cells, mesenchymal stromal/stem cells, and induced pluripotent stem cells [2][3]. This array of orthobiologics has enabled researchers to target different diseases at a cellular level and has bestowed physicians with growing potential in the ever-expanding field of regenerative medicine [4].

2. Embryonic Stem Cells (ESCs) and Induced Pluripotent Stem Cells (iPSCs)

Embryonic stem cells (ESCs) are isolated from the inner cell mass of the blastocyst during the pre-implantation period. Two of the most refined properties of these cells are their capacity to infinitely proliferate without differentiation and, at the same time, retain the potential to generate all three germ layers [5]. These properties are, respectively, termed self-renewal and pluripotency.

The tenogenic potential of ESCs has been successfully demonstrated both in vitro and in animal models, but the transition to clinical trials still requires further investigation of their tumorigenic potential [6][7][8]. In a large animal model trial, ESCs showed improved healing in collagenase-induced flexor tendinitis [9]. Tissue architecture, tendon size, tendon lesion size, and fiber patterns of the tendons were significantly improved on histologic sections and ultrasound in the horse-treated group, even without in vitro pre-differentiation.

On the other hand, how to differentiate ESC into tendon lineage is the key point. Some authors have suggested that a stepwise differentiation of human ESCs (hESC) into MSCs could potentially allow these multipotent cells to form tendon-like tissues, with the advantage of avoiding teratoma formation [10]. In vitro and in vivo studies showed that ESC-differentiated MSCs (hESC-MSCs) markedly presented tenocyte-like morphology and expressed tenocyte-related gene markers such as COL-1 and -3 and SCX [11]. Additionally, tendon repair treated with hESC-MSCs showed better ectopic tendon regeneration and mechanical properties than did controls, with hESC-MSCs remaining viable for longer periods [11].

Similar results were shown by the use of hESC-MSCs incorporated within collagen sponge scaffold to promote tendon regeneration [12]. hESC-MSCs exhibited tenocyte-like morphology and also positively presented tendon gene markers (COL-1 and -3, Epha4, and Scleraxis). After in vivo transplantation and under the mechanical stimulus, the tissue displayed better alignment and configuration of the collagen fibers and superior mechanical characteristics [12]. Other potential benefits of hESC-MSCs transplantation in tendon pathology is the in situ environment-modifying effects,

indirectly favoring tissue regeneration. However, the use of ESCs may still be limited by ethical concerns since a sacrificed embryo is needed [13][14][15][16].

In this sense, the later discovery of Induced Pluripotent Stem Cells (iPSCs) can potentially solve the ethical problem of ESCs, in which researchers generated iPSCs from totally differentiated cells through specifically transcription factors delivered by retroviruses or by miRNA delivered directly to generate integration-free iPSCs [17][18]. Human iPSCs have been shown to better repair rat patellar tendon window defects in comparison to non-iPSCs treated tendons, demonstrating by macroscopical, histological, and biomechanical analysis that human iPSC promotes tendon repair in animal models [19]. Tenocytes derived from human iPSC can provide a therapeutic option for tendon injury. iPSC-tenocyte grafting contributed to motor function recovery after Achilles tendon injury in rat models via paracrine effect and engraftment. [20] While the use of ESCs and iPSCs is progressively growing, it has not been reported in human studies of tendon tissue engineering yet. On the other hand, recent progress in culturing these cells to properly differentiate them brings great expectations for the use of human-ESC based therapy in the near future [21][22].

3. Mesenchymal Stromal Cells (MSCs)

MSCs possess self-renewal potential and have the ability to differentiate into specific mature cell lineages [23]. They are characterized by a set of specific cell surface cluster differentiation markers (CD), which are known to express a range of cell-lineage-specific antigens which differ depending on culture preparation, duration, or plating density [24][25]. MSCs can manipulate and control their local microenvironment due to the paracrine and autocrine effects [26]. MSCs do not trigger aggressive immunogenic episodes and they can be easily isolated, facilitating allogenic transplantation in appropriate circumstances. Therefore, these cells may be considered immune evasive, however, the regenerative effects of MSCs in cellular-based therapies are usually more associated with their homing and engraftment abilities in target tissues [27]. Furthermore, MSCs have a rather short life span and are ultimately phagocytized by monocytes, subsequently stimulating the production of T-regulatory (Treg) cells, thereby maintaining homeostasis and self-tolerance [28][29]. MSCs act as trophic mediators to attenuate escalated apoptosis, fibrosis, and inflammation whilst stimulating cell proliferation and differentiation via paracrine and autocrine signaling [30].

Bone marrow-derived MSCs (BM-MSCs): The cellular components of bone marrow can be divided into non-hematopoietic cells (pericytes, endothelial cells, osteoblasts, adipocytes, and Schwann cells) and hematopoietic cells (neutrophils, lymphocytes, megakaryocytes, monocytes, and osteoclasts) [31]. Additionally, there is also the presence of the hematopoietic stem cells (HSC) and mesenchymal stromal/stem cells (MSCs), the two major adult stem cell types found in this tissue. The MSCs present in bone marrow contain a potent anti-inflammatory cytokine known as interleukin-1 receptor antagonist (IL-1Ra) [32]. IL-1Ra also reduces matrix degradation, MMP-3, and TNF-α gene expression, PGE2 secretion, chondrocyte apoptosis, and enhances collagen deposition. [33] Collectively, the effects elicited by IL-1Ra are of great clinical value as they can bring significant pain alleviation to the patient and improve the state of prolonged tissue injury inflammation, especially in tendinopathies. Intratendinous administration of BM-MSCs in rabbit model AT improves biomechanical (improved biomechanical modulus) and histological (improved collagen fibers organization) scores in the early phase of tendon healing [34]. In a rat model of Achilles tendon rupture, BM-MSCs show superior tendon healing potential than PRP in terms of histological, biochemical, and immunohistochemical scores [35]. In a rat model of Achilles tendon rupture, an increased expression of Tenascin-C was observed equally in the groups treated with tendon stem cells (TSCs) and BM-MSCs but TSCs exhibited higher regenerative potential than BM-MSCs. Hence, TSCs are the better sources of stem cells for tendon regeneration [36]. Under ultrasound (USG) guidance, autologous bone marrow aspirate concentrate (BMAC) injected intralesionally into the mid substance tendinopathic region of Achilles in a female patient with chronic MRI confirmed AT exhibited less pain with the normal activity of daily living (ADL) after 2 months of post-intervention. Improvement in relative strength intensity was observed in T1W-MRI images after 10 weeks post-intervention [37]. Improved Achilles tendon rupture scores were observed with autologous BMAC augmentation in Achilles tendon rupture [38]. BM-MSCs promote early rehabilitation, lower incidence of re-rupture, improvement of pain scores, and amelioration of tendon structure and strength, without the occurrence of serious complications [38][39][40][41][42][43][44]. van den Boom et al. derived level 4 evidence for BMAC augmentation in Achilles tendon repair in terms of improved PROMs and absence of re-tears in 2.5 years follow-up [45]. However, more robust data are still required to further support the efficacy and safety regarding the clinical administration of BMA for AT, more specifically.

Adipose tissue-derived MSCs (AD-MSCs): In recent years, adipose tissue and its derivatives have also received a considerable amount of attention from the scientific community by presenting itself as a novel and potential cell source for tissue engineering and regenerative medicine [46][47]. A total yield of stem cells in adipose tissue was approximately 40 times greater than bone marrow [48][49][50]. SVF, a product of adipose tissue, carries a wide variety of cells, including endothelial cells, preadipocytes, type 2 macrophages, T cells, pericytes as well as mesenchymal stromal/stem and

progenitor cells [46][47][51]. The application of SVF for the treatment of tendinopathies, specifically, yields satisfactory regenerative outcomes [52][53][54][55]. An in vivo study with SVF and AD-MSCs demonstrates the maintenance and induction of tendon fiber organization [53]. Usuelli et al. demonstrated that the intratendinous SVF injection exhibited faster recovery results at just 15 days after treatment for AT [52]. Piccionello et al. revealed the significant findings in the ovine model of tendinopathy [53]. Matrix composition and collagen deposits in treated tendons are significantly enriched, and neo-angiogenesis is improved within the lesion sites and it was concluded that the reorganization of tendon fibers is just as important as the proliferation, differentiation, and immunomodulatory capacities of SVF cells [53]. In a collagenase-induced AT mice model, AD-MSCs facilitate neovasculogenesis, upregulate tendon repair, downregulate ectopic ossification, and inhibit inflammation in Achilles tendon healing [56]. At 3 months follow-up, the improvement of AOFAS and FADI scores was observed in surgically managed Achilles tendon tears with micro fragmented adipose tissue (M-FAT) [57]. M-FAT downregulates the expression of type 3 collagen and metalloproteases-1 in a significant manner and upregulates the production of VEGF, IL-1Ra, and IL-6 in an in vitro model of tendinosis [58]. Tenogenically differentiated AD-MSCs upregulate the gene expression of COL-1 and -3, scleraxis, decorin, tenascin-C, and tenomodulin, modulate cytoarchitecture, and improve the histological score, organization of collagen fibers, recovery of elastic modulus, and tensile load of tendons over time in Achilles tendon repair in vivo [55]. SVF derived from adipose tissue pose superior results in terms of clinical and functional outcome in AT when compared with PRP [59][60]. van den Boom et al. derived level 3 evidence for allogenic AD-MSCs in the management of AT when compared with PRP [45]. Although the basic science studies in the literature have already revealed positive outcomes, there is still a great need for more robust clinical data to further validate the efficacy and safety of the application of adipose tissue-derived products in tendon healing.

4. Acellular Therapy in Achilles Tendinopathy

Acellular therapy marks the application of nanomedicine principles in the management of musculoskeletal disorders. The micromolecules from cells and tissues play a significant role in targeting the desired site with therapeutic applications. The most commonly used acellular biological products are platelet-rich plasma (PRP), concentrated growth factors, and exosomes from various sources.

4.1. Platelet-Rich Plasma (PRP)

Chronic tendinopathy creates a pro-inflammatory environment and hinders the healing cascade due to precarious blood supply and comparably slower cell turnover in the case of tendons [61]. Literature evidence supports that PRP has targeted therapeutic applications in musculoskeletal disorders by enhancing regeneration of diseased or degenerated tissues. By concentrating platelets, the growth factors are released from alpha granules of platelets which enhance the natural healing cascade. PRP contains WBCs and chemokines, which regulate inflammatory responses [62][63]. The type of PRP to be used depends upon the disease condition and targeted site in the body to have maximum benefits outweighing the risks. Yoshida et al. [64] demonstrated that the combination of leucocytes with platelets in an ACL fibroblast culture promoted significant increases in type I and type III pro-collagen gene expression, collagen production, and cellular proliferation. The administration of PRP accelerates and hastens neovascularization and stimulates the potentiation of the resident stem cells and the subsequent restoration of injured tissue. Upon activation of PRP, numerous pockets of growth factors and cytokines release in the desired site and exert anabolic and anti-inflammatory actions by potentiating various cells and their secretomes [65]. PRP potentiated the differentiation of TSCs to mature tenocyte by increasing the proliferation and collagen production [66]. The platelets in PRP stimulate macrophages and fibroblasts to repair the damaged collagen fibrils of the tendon and enhance neovasculogenesis and collagen organization in the injured tendon [67][68].

The outcome of PRP injection for AT demonstrated decreased vascularity and changes in the tendon thickness as reported in a few studies [69][70][71][72] whereas a few researchers have provided controversial data stating the increased tendon thickness after 3 months follow-up [73][74]. Filardo et al. demonstrated a stable outcome to a medium-term follow-up with repeated intra-tendinous PRP injection in recalcitrant AT [75]. Gaweda et al. reported a significant decrease in tendon thickness and hypoechoic lesions along with the normalization of peritendineum in AT when treated with PRP [76]. Deans et al. demonstrated a significant clinical improvement in recalcitrant AT with a single dose of autologous conditioned serum along with regular exercises and therapeutic ultrasonography [77]. Monto et al. reported significant USG and MRI changes in Achilles tendon substance in pre-and post-PRP treatment in AT [78]. de Vos et al. reported no greater improvement in pain and activity, when AT is treated with eccentric exercises and PRP [70]. In a meta-analysis with seven clinical trials by Chen et al., no trial has demonstrated that PRP improves either clinical or functional outcomes in AT and hence RCTs were expected to test the hypothesis [79]. In a meta-analysis, Liu et al. reported limited evidence support that PRP is not a superior treatment to placebo management in chronic AT [80]. Zhang et al. reported no improvement in VISA-A scores, tendon thickness, or color Doppler activity in AT with PRP [81], whereas Madhi et al. demonstrated a significant

improvement in VISA-A scores with the usage of PRP in AT [82]. PRP is found efficacious in young to middle-aged patients with non-insertional AT compared to old aged patients. This data is ascribed to biomechanical differences in the tendinous substances [83]. Townsend et al. formulated post-PRP protocol for AT by the initiation of stretching exercises by 2 weeks after injection and then full return to play was advised after 6 weeks of PRP injection [84]. Despite controversy in the literature, many studies share a common ground in the sense that PRP consistently presents itself as a safe and effective biological agent for the amelioration of both chronic and acute Achilles tendon injuries, with significant improvements in pain and functional outcomes [69][80][85][86].

Exosomes

The sourcing of exosomes (Exos) presents a major challenge in scaling production in terms of commercialization and therapeutic efficacy of clinical applications. Exos can be derived from either cellular (hematopoietic cells, mesenchymal stromal cells, immune cells and tissues in the form of organs) or noncellular sources (body fluids). Production of large amounts of Exos is expensive, technically demanding, and ethically challenging. Local administration of Exos in the bone–tendon interface downregulates the genes responsible for pro-inflammatory cytokines, excessive scar formation, cellular apoptosis, and M1 macrophages and upregulates the genes responsible for anti-inflammatory cytokines and ECM synthesis [87][88]. MSC-Exos modulate collagen organization and macrophage polarization, proliferate tenocyte and fibroblast cells, and inhibit tenocyte adhesions in tendon disorders [89]. BM-MSC-derived Exos enhance the healing of the tendon–bone interface by regulating M2 macrophage polarization [90]. Administration of TSC-derived Exos into rat AT leads to downregulation of MMP-3 gene expression with upregulation of TIMP-3 and COL1A1 gene expression, balancing of ECM in the tendon, and potentiates ethnogenesis of TSCs [91]. TSCs regulate immunomodulation in tendons through c-Jun N-terminal kinase and STAT-3 signaling [91]. TSC-derived Exos containing TGF-β enhance the migration, proliferation, and differentiation of TSCs through Smad2/3 and ERK1/2 signaling pathways [92]. Tenocyte-derived Exos express higher levels of CD-9 and -61, TSG-101, COL-1 and -3, TNMD, Shc, p-ERK1/2, and integrin β1 to enhance the proliferation of MSCs [93].

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