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Tendon-derived stem cells as a new cell source for tendon tissue engineering

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1. ABSTRACT

Tendon injuries are very common in occupational and athletic settings, and the elderly population. Tendons repair and regenerate slowly and inefficiently *in vivo* after injury. The limited ability of tendons to self-repair and the general inefficiency of current treatment strategies have intensified the need for an effective therapeutic approach. Tendon-derived stem cells (TDSCs) have recently been identified within tendon tissues. TDSCs exhibit universal stem cell characteristics, such as clonogenicity, a high proliferative capacity, multi-differentiation potential, non-immunogenicity, and immunosuppression. As a result, implanting TDSCs at damaged sites within tendons may be an effective way for tendon regeneration. This review summarizes the properties of TDSCs and discusses the advantages of its use in tendon tissue engineering.

2. INTRODUCTION

Tendons connect bone with muscle and are essential for transmitting forces to produce movement. Tendons are characterized by low vascularization and a low cell density. Tenocytes produce the extracellular matrix (ECM), which consists mainly of collagen types I (> 90% of total collagen) and III and several proteoglycans, which is responsible for the high tensile strength. Tendon injuries are a common problem in occupational and athletic settings that involve repetitive mechanical loading on tendons (1). It has been demonstrated that there are approximately 30 million annual tendon and ligament injuries worldwide, at least 200,000 people in the United States undergo treatment for injuries to tendons or ligament tissues each year (2). Healing of injured tendons is slow, especially when the injury is substantial or when rupture with tendon retraction

occurs. Tendon healing also forms scar tissues, which impairs joint function and makes the healed tendons susceptible to re-injury as the scar tissue has inferior mechanical properties (3, 4). There are a number of methods to treat tendon injuries, but none are completely effective. Currently, enormous interest exists in regenerative medicine. In recent years, bone marrow mesenchymal stem cells (bMSCs) have been used to regenerate functional tendons and ligaments. It was not until 2007 that Bi *et al*. (5) first showed that human and mouse tendons harbor a unique cell population, the so-called tendon stem/progenitor cells or tendon-derived stem cells (TDSCs), demonstrated clonogenicity, self-renewal, and multi-differentiation potential. Like bMSCs, isolated TDSCs are able to regenerate tendon-like tissues after extended expansion *in vitro* and transplantation *in vivo*, and exhibit many advantages against bMSCs. As a result, TDSCs may be a good cell source for tendon tissue regeneration. In this review, we focus on the properties of TDSCs and discuss the application of TDSCs in tendon tissue regeneration.

3. TDSCS ISOLATION AND CULTURE

TDSCs have been isolated and cultured from adult human (5), mouse (5), rabbit (6), rat (7), horse (8), and fetal human (9) tendons. The TDSCs are prepared from tendon tissues based on preferential attachment and potent clonal expansion over the majority of tendon resident cells (5). Firstly, the mid-substance of the tendon is obtained, the peritendinous connective tissue is removed, cut into pieces, and digested with type I collagenase and dispase. Then, the digestates are centrifuged and resuspended in low-glucose Dulbecco's modified Eagle medium, supplemented with 10% fetal bovine serum, 10 U/mL of penicillin, 100 mg/ml of streptomycin, and 2 mML-glutamine (complete basal culture medium). After diluting the suspension to 10 cells/ml, the cells are plated at the optimal low density, and cultured at 37°C in 5% carbon dioxide to form colonies. We also use 70-μm pore-size nylon filters to recover the isolated cells. On day 2 after initial plating, the cells are washed twice with phosphate-buffered saline to remove the non-adherent cells. A portion of tendon-derived cells attach to the plate and remain quiescent for 3–5 days before undergoing rapid division to form colonies (9). On days 7–10, the cells are trypsinized and mixed together as passage 0.

The cells isolated are most likely a mixture of stem and progenitor cells, which are heterogeneous with respect to clonogenicity, multi-differentiation potential, and self-renewal capability, as colony size and cell density of TDSCs vary greatly, TDSCs did not completely differentiate into the same type of cells under the same induction media, and not all individual cells are noted to express the same stem cell markers such as Oct-4 and SSEA-4 (6). The shape of TDSCs also varies as a function of the species, tissue origin, cell passage number, and confluence of the culture.

The most effective way to culture TDSCs is unknown, and recent strategies to culture TDSCs are not completely effective. Because the tendon milieu is relatively oxygen (O_2)-deficient, cells from these tissues are usually cultured with 20% O_2, which is "physiologic hyperoxia" (10, 11). Research suggests that hypoxia is advantageous for efficient expansion of hTDSCs *in vitro*, as TDSCs exhibit higher clonogenicity and cell proliferation at 2% than 20% O_2 tension. Moreover, the 2% group expressed higher levels of the tendon-related marker, tenomodulin (TNMD), and lower but reversible multi-differentiation potential compared with the 20% group. At the same time, hypoxia had no apparent effect in immunophenotypes, senescence-associated β-galactosidase activity, and the collagenous or non-collagenous protein production ratio (12). Previous studies have shown that a lower seeding density favors the enrichment and proliferation of stem cells (13, 14), which is similar to TDSCs. Plating cells at 50 cells/cm^2 was shown to yield the highest number of single cell colonies per tendon-derived nucleated cell compared with the densities of 500, 5000, and 50,000 cells/cm^2. Cells seeded at a lower density (100 cells/cm^2) proliferated much faster than cells seeded at 500 or1000 cells/cm^2; it is unlikely that a nutrient deficiency was a limiting factor for cell growth (7). Another experiment suggested that colonies at seeding densities of 50 cells/cm^2 formed well because the colony size was not affected by colony-to-colony contact inhibition and the greatest number of colonies per tendon-derived nucleated cells was obtained (15). However, Tan *et al*. (16) reported the optimal initial cell density for the isolation of TDSCs from rat patellar tendon was 500 cells/cm^2. Nevertheless, all of the researches presented above suggested to us that a lower seeding density appears better for TDSCs proliferation, whether 50, 100, or 500 cells/cm^2. Moreover, with the use of a dynamic bioreactor with an optimal supply of nutrients, removal of wastes and regulation of the microenvironment can improve the process (12).

4. CHARACTERISTICS

4.1. Immunophenotypic prolife

Compared with other stem cells, no single marker can identify TDSCs; rather, a combination of factors must be used. TDSCs express many markers, like bMSCs, but the markers are not identical (5). TDSCs have been reported to express Stro-1, CD44, CD146, CD105, CD90, nucleostemin, Oct-4, SSEA-4, Sca-1, and CD73, and are negative for CD18, CD45, CD117, CD34, CD106, CD31, Flk-1, and CD144 (5-7, 15-20). In like manner, bMSCs express many markers, such as CD18, CD 144, and CD 106, and are negative for stro-1, CD90.2, and Sca-1. A summary of the phenotypic markers in TDSCs isolated from different species and bMSCs is shown in (Table 1). At the same time, there is higher colony-forming ability and proliferative and multi-lineage differentiation potential upon induction, and higher osteogenic, chondrogenic, and tenogenic marker expression in the basal state in TDSCs compared to bMSCs (5, 19, 21). Consequently, it is likely that TDSCs may be a unique cell type distinct from bMSCs.

Nevertheless, the possibility that TDSCs and bMSCs are different stages of common MSCs cannot be

Table 1. Expression of phenotypic markers in TDSCs isolated from different species and in bMSCs

Species	Human (5, 9, 18)	Mouse (5)	Rat (7, 15-18, 20)	Rabbit (6)	bMSCs (48, 62-64)
Stro-1	+				-
CD44	+	+	+		+
CD146	+				+
CD105	+				+
CD90	+		+		+
CD90.1			+		
CD90.2		+			-
nucleostemin		+	+	+	-
Oct-4			+	+	+
SSEA-4			+	+	+
Sca-1		+			-
CD18	-	-			+
CD45	-	-	-		-
CD117	-	-			-
CD34	-	-	-		-
CD106	-		-		+
CD31			-		-
Flk-1		-			+
CD144		-			+
CD73	+		+		
CD19	-				
CD14	-				

excluded. TDSCs may be imprinted under the influence of a local environmental milieu so that they are more likely to produce tendon and junctional tissues (19). A recent study suggested that perivascular cells within tendon tissues expressed tendon cell markers (scleraxis, collagen type I, collagen type III, and smad8) and stem/precursor cell markers (CD133, Musashi-1, Nestin, CD44, and CD29), in addition to the pericyte-associated marker α-SMA. Moreover, perivascular cells exhibit clonogenicity and multi-lineage differentiation potential, thus suggesting that the perivascular niche is a source for tendon precursor cells and the perivascular cell appears to be a mixture of tenocytes and TDSCs (22). At the same time, previous studies have suggested that all MSCs are pericytes that gradually assume tissue-specific phenotypes under the influence of the local niche (23, 24). Interestingly, Rui *et al.* (7) reported that TDSCs are pericytes as well because rat TDSCs expressed α-SMA. Moreover, Scx delivery resulted in the direct programming of bMSCs into tendon progenitors (25). In conclusion, we suggest that a "maturation gradient" exists in the tendon from MSCs, followed by TDSCs, perivascular cells, and finally tenocytes.

The immunophenotypic prolife may be influenced by age. Zhou (17) reported that TDSCs maintain stemness because TDSCs stain positively for three stem cell markers (nucleostemin, Oct-4, and SSEA-4) independent of age; however, aged TDSCs express lower levels of CD90.1 than younger cells, but higher levels of CD44, which is usually down-regulated in healing of many tissues. Thus, increased CD44 expression in aged TSPCs may contribute to reduced repair capacity in TDSCs with age (17).

4.2. Cell proliferation

TDSCs, similar to other MSCs, proliferate faster than terminally differentiated cells *in vitro*. Human and mouse TDSCs proliferate faster than bMSCs isolated from the same source (5). TDSCs isolated form different tendons in the same individual also have different proliferative potential (26).

The proliferation of TDSCs is also affected by age. Aged tendon tissues have fewer TDSCs and aged TDSCs have a longer population doubling time (PDT; 17). Analysis of the cell cycle phase distribution has shown that aged TDSCs contain an arrest in G2⁄M which could result from accumulated genetic and/or epigenetic damage. Cited2 is a transcription factor implicated in the control of growth and senescence in several cell types (27-30). Previous studies have suggested that Cited2 expression in aged TDSCs is reduced, consistent with positive roles for Cited2 in TDSC self-renewal (17). Additionally, differences in apoptotic rates between young and old TDSCs could also contribute to the observed disparities in population size (17).

4.3. Differentiation potential

TDSCs are able to differentiate into tenocytes, chondrocytes, osteocytes, and adipocytes *in vitro*, and form tendon-, cartilage-, bone- and tendon–bone junction (TBJ)-like tissues in human and animal models (5-9).

The ability of TDSCs to differentiate into tenocytes is also diminished by age. There are age-related declines in mRNA expression of scleraxis (Scx) and TNMD, two tendon lineage-related molecular markers in TDSCs at the basal level and upon induction by transforming growth factor (TGF)-beta3 (17). Aged TDSCs form adipocytes more readily than younger cells, and express higher levels of adipogenic markers following induction. Young and old TDSCs show no apparent difference in the ability to form osteocytes or chondrocytes (17), which may help to explain the higher levels of adipose in older tendons. Furthermore, the coordinated expression of Cited2 and Scx suggests that Cited2 may also regulate TDSCs differentiation (17).

4.4. Physiologic niche

Stem cells have limited functions outside a niche. The stem cell niche has been defined as a specialized microenvironment that houses stem cells and maintains a balance of quiescence, self-renewal, and cell-fate

commitment. The stem cell niche is a three-dimensional structure composed of cells, cytokines, and ECM (31, 32). Although most of the identified niches are cell-based, the ECM may also be important in the regulation of TDSCs, as TDSCs reside in between the long parallel chains of collagen fibrils and are surrounded predominantly by ECM (5). Specific components of the ECM-rich niche control the fate of TDSCs by modulating the bioactivities of growth factors and cytokines to which ECM proteins often bind. For example, a deficiency in the ECM proteins, Bgn and Fmod, activates BMP2 signaling through Smad1-Smad5-Smad8 pathway and increases the expression of Runx2. As a result, Ca^{2+} accumulation and alkaline phosphatase activity is substantially increased, thus facilitating ectopic ossification. In addition, tendons appear more translucent and are significantly thinner and more cellular than control tendons. Collagen fibers within the tendon are disorganized with large gaps inside (5). Another study showed that TGF-beta signaling is essential for maintenance of TDSCs and may mediate the recruitment of new tendon cells (33). Insulin-like growth factor-I (IGF-I) and fibroblast growth factor-2 (FGF-2), along with TDSCs, supplement *in vitro*-enhanced cell persistence and proliferation and matrix synthesis (34, 35). The intra-articular injection of the analgesics, ropivacaine and bupivacaine, post-operatively, significantly reduces cell viability, metabolism, and apoptosis in TDSCs in a dose-dependent fashion, while morphine has no such affect (18). As a result, we hypothesize that ECM makes a significant contribution to the maintenance of TDSC function. Further studies should determine the constituents of ECM and how the constituents regulate the fate of TDSCs.

In addition to ECM, mechanical signaling plays an important role in tendon physiology (36). In paralyzed embryos, tendon formation occurs, but is markedly inhibited (37). During development and in adults, long-term exercise leads to stronger tendons, whereas immobility or injury leads to tendon weakening and metaplasia (38). Thus, it is generally believed that mechanical loading plays a dominant role in tendon development and tendinopathy. Mouse treadmill running exercise induces an anabolic effect on tendons by enhancing TDSC proliferation and increasing TDSC-related cellular production of collagen (39). *In vitro*, TDSCs are aligned along the direction of mechanical stretching and slightly better cell alignment (8% compared to 4%), as well as longer stretching time (40). Moreover, using a multi-potent MSC line, Scott *et al*. (41) reported that cyclic loading results in a greater increase in tenocyte gene (Scx and Col1a1) expression than static loading *in vitro*. The same results were generated when the strain level was increased, inclusion of a 10 s rest period, and an increase in the number of repetitions. Nevertheless, excess mechanical loading may be harmful to TDSCs. Low mechanical stretching at 4% promotes differentiation of TDSCs into tenocytes, whereas large stretching at 8% induces differentiation into adipogenic, chondrogenic, and osteogenic lineages (26). A repetitive tensile load increases protein and gene expression of BMP2 in TDSCs, and promotes osteogenic differentiation, which may account for ectopic calcification in calcifying tendinopathy (40). In another study, high levels of PGE2 were produced in response to repetitive mechanical loading *in vivo*, and the presence of high levels of PGE2 were shown to have a detrimental effect on tendons by decreasing TDSC proliferation and inducing stem cells to differentiate into adipocytes and osteocytes in an apparent dose-dependent fashion (42). Furthermore, an *in vitro* experiment showed that PGE2 treatment of TDSCs suppressed cell proliferation and induced osteogenic differentiation, which was mediated by BMP-2 produced in culture (43). Shi et al. (15) reported that uniaxial mechanical tension promotes osteogenic differentiation of TDSCs with increased Runx2 expression via the Wnt5a-RhoA pathway.

In addition to ECM proteins and mechanical loading, the nano- and micro-structure of ECM, tenocytes, neural input, vascular input, and physiologic factors, such as oxygen tension and metabolic products of tissue activity, are likely to be important niche components which regulate the fate of TDSC. Specifically, aligned electrospun nanofibers provide an instructive microenvironment for TDSCs to differentiate into a teno-lineage, and can resist the power of an osteogenic induction medium compared to a random nanofibrous scaffold (9). Tenocytes produce collagen, which is the main content of ECM and makes up the frame of the tendon. Tenocytes also secrete many cytokines and growth factors that are necessary for the function of TDSCs; however, how tenocytes affect the fate of TDSCs is unknown, and additional studies should be conducted. The stem niche is a complex mixture; indeed, we know very little about the stem niche, which limits the application of TDSCs in tendon regeneration.

5. TDSCS FOR TISSUE REPAIR

The ability to use small portions of stem cells for *in vitro* expansion, and then use to form tendon tissue *in vivo* offers a new strategy for improving the current method of tendon repair. A number of studies have been conducted to determine the use of bMSCs in tendon regeneration, but few studies have been conducted with TDSCs. Indeed, TDSCs have many advantages over bMSCs with respect to tendon tissue engineering.

5.1. Advantages against BMSCS

The number of stem cells required for tissue repair in the clinical setting is often > 10^{10} cells (44). For tendon repair, previous studies involving rodents have shown that at least 10^6 bMSCs are required for tissue repair (45, 46). Thus, it is not surprising that a greater number of cells are needed for tissue repair in humans; however, only 0.001–0.01% of the total number of nucleated cells in human bone marrow aspirates are bMSCs (47, 48). Moreover, the number of bMSCs obtained depends on the volume of the aspirate, while the concentration of bMSCs obtained per ml decreases with an increase in the volume of the aspirated marrow because of dilution of the bone marrow sample with peripheral blood (49). In contrast, tendon contains a larger percentage of TDSCs. Mouse patellar and human hamstring tendons have been reported to contain 3–4% TDSCs (5). Rat flexor tendons contain 1–2% TDSCs (7). Of human fetal Achilles TDSCs at passage

2, 5–6% are able to form colonies (9). As a consequence, the percentage of stem cells in tendons exceeds the percentage in bone marrow aspirates by at least three orders of magnitude. What's more, TDSCs are more proliferative in culture and require a shorter time to generate a clinically-relevant number than bMSCs (19, 34).

Moreover, TDSCs exhibit higher tenogenic, osteogenic, chondrogenic, and adipogenic differentiation markers and differentiation potential towards tenocytes, osteocytes, chondrocytes, and adipocytes upon induction than bMSCs (5, 19, 21). Bi *et al*. (5) reported that mouse TDSCs express higher mRNA levels of Scx, Comp, Sox9, and Runx2 than bMSCs, while human TDSCs express a higher level of TNMD than human bMSCs. Murine and human TDSCs accumulate Ca^{2+} more rapidly (approximately four-fold) and form more calcium nodules compared to bMSCs upon osteogenic induction. In addition, TDSCs express a higher level of BMP receptors and exhibit greater osteogenic differentiation with and without BMP-2 stimulation (21). TDSCs cultured *in vitro* produce more ECM supplemented with and without IGF-I than bMSCs (35). Thus, TDSCs may be more suitable for soft tissue regeneration. Of further importance, ectopic bone and tumour formation have been reported in special circumstances after transplantation of bMSCs (50, 51), thereby exacerbating the tendinopathy. These results suggest that TDSCs may be a promising therapeutic cell source for tendon regeneration compared with bMSCs.

5.2. *In vitro* passing

A large number of TDSCs are required for therapeutic application in tissue repair. Therefore, *in vitro* cell expansion and optimization of culture conditions for large-scale production of TDSCs is crucial. At the same time, the cells have to preserve stem cell-related properties during prolonged *in vitro* passaging. Previous studies have shown that bMSCs enter senescence and begin losing stem cell characteristics during *in vitro* passaging, which affects the application of bMSCs for tissue regeneration (52, 53). It is important to determine whether or not the same conditions will apply to TDSCs. In a recent study, β-galactosidase activity increased, while stem cell-related marker expression and the multilineage differentiation potential decreased in TDSCs with *in vitro* passaging; this occurred despite the increase in the colony number and proliferative potential of TDSCs during subculture (up to P30; 16). Immunocytochemical staining showed that TDSCs in culture for > 3 months at passages 10–13 maintained stemness with expression of Oct-4, SSEA-4, and nucleostemin. For TDSCs at subsequent passages (> 12), the PDTs are increased, indicating that the cells are in a senescent state (6). The surface expression of CD90 and CD73 is also down-regulated during passaging, consistent with studies involving bMSCs; however, the significance of this observation remains unknown (16). Moreover, Tan *et al*. (16) reported a loss of adipogenic, chondrogenic, and tenogenic differentiation capacity of the cells with increased commitment of the cells toward the osteogenic lineage during subculture. As a result, it appears that *in vitro* passing damages TDSCs; thus, using a small number of TDSCs for expansion with incessant passing is impossible. In another study, cells were shown to exhibit different cell morphologies at P0 and P1; however, a homogeneous population of fibroblast-like cells was observed at P3 (7). The findings suggested that cells from P3 are suitable for *in vitro* stem cell differentiation and tissue engineering studies (7); however, the observations were limited to P3, and what would occur during subsequent passing is unknown.

5.3. How TDSCs achieve regeneration?

TDSCs significantly enhance the early stage of tendon healing, as indicated by the increase in collagen production, and improvement in cell and collagen fiber alignment, collagen birefringence (typical of tendons), and biomechanical properties of regenerated tissue. Moreover, no ectopic bone formation was observed up to week 4 (5, 54, 55). Nevertheless, how TDSCs promote tendon recovery is unknown. The proliferation and differentiation of TDSCs into tenocytes may play an important role. In other studies it has been shown that transplanted cells contribute to tendon healing by producing tropic paracrine factors that promote healing, rather than direct differentiation to tenocytes as the number of TDSCs decrease with time (54, 56).

5.4. Ways to enhance the regeneration

With a change in the composition of the stem niche, we can successfully proliferate and induce TDSCs into tenocytes; therefore, we are able to design new stem cell therapeutics for the treatment of disease. However, little is known about the stem niche. As discussed above, TGF-beta signaling, IGF-I, FGF-2, and mechanical loading play an important role in the application of TDSCs. Moreover, platelet-rich plasma (PRP) contains various types of growth factors, such as platelet-derived growth factor (PDGF), epidermal growth factor, TGF-beta1, IGF-I, and receptors (e.g., TGF-beta1 receptor), which are involved in tendon healing (57-59). With the treatment of PRP, TDSCs differentiate into tenocytes, which in turn produce abundant collagen. The combination of TDSCs and PRP has synergistic effects on tendon healing under loaded and unloaded conditions; the loaded group has a higher level of collagen I and III expression than the unloaded group (1, 20). Zhang *et al*. (55) developed a soluble engineered tendon matrix (ETM) from decellularized tendon tissues which retained considerable bioactivity in terms of promoting TDSCs proliferation and preserving the stemness of TDSCs compared to plastic surfaces. The combination of ETM and TDCs also promoted the differentiation into tenocytes and was able to form more, and better, tendon-like tissues *in vivo* compared with planting TDSCs alone; no evidence showed that non-tendinous tissues were formed (55). In contrast, with the use of Matrigel, a basement membrane product, not only tendon-like tissues, but also fatty, cartilage-like, and bone-like tissues were formed, indicating that Matrigel is not an ideal matrix for tendon-like tissue formation by TDSCs (5, 6).

Considering the physiologic properties of tendon tissue, an application technique via scaffolds and matrix with the capability of cell seeding and adhesion would be

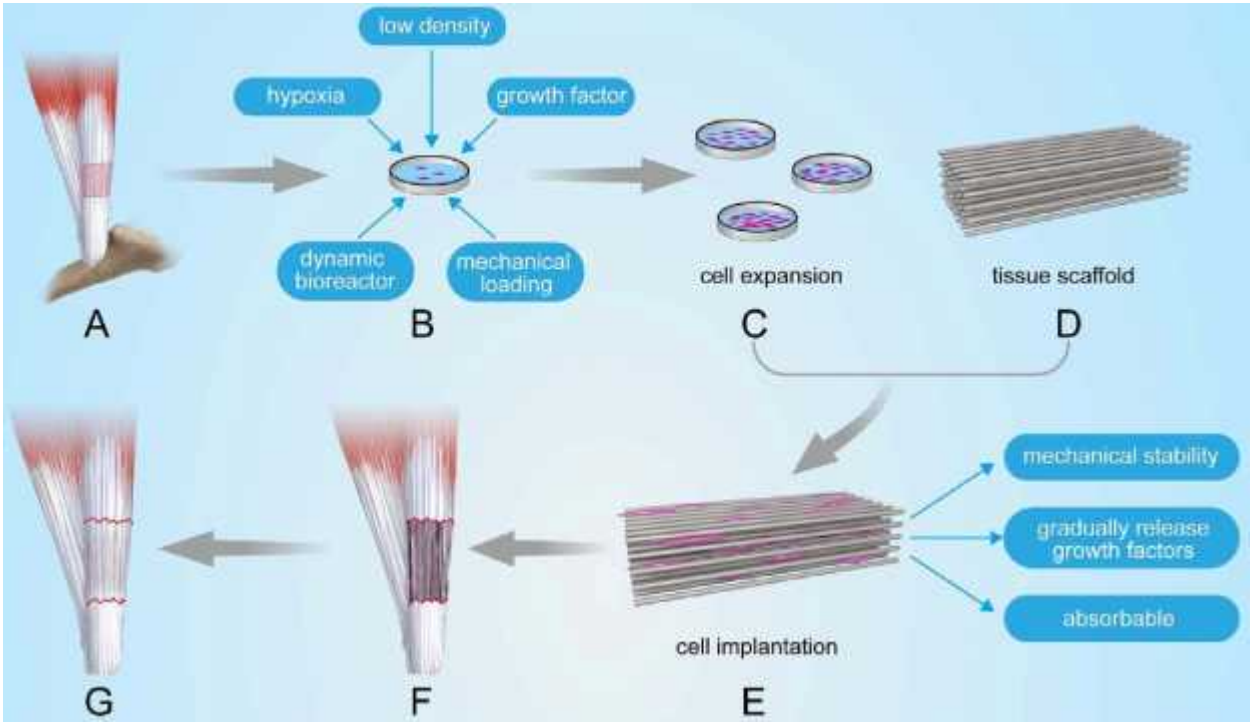

Figure 1. The process by which TDSCs promote tendon regeneration via scaffolds.

ideal (60). The scaffolds should provide short-term mechanical stability of the transplant before degradation with time, so that the injured tendon exhibits normal function and the TDSCs are exposed to mechanical loading, which will promote the proliferation, differentiation, and secretion of collagen. Moreover, the scaffolds, or the matrix, should be able to deliver and gradually release the growth factors or the nutrition that drives the process of cell differentiation and maturation. In addition to acting as a carrier for stem cells and growth factors, scaffold elasticity, stiffness, composition, and the matrix micro- nano-structure can also modulate cellular behaviour, such as cell alignment, migration, proliferation, and direct stem cell differentiation. The process of TDSCs to promote the tendon regeneration via scaffold is represented in (Figure 1). Few studies have been conducted to explore the application of scaffold upon TDSCs. In the current study, TDSCs exhibited universal stem cell characteristics; non-immunogenicity, immunosuppression, and proliferated well on TDSC-seeded knitted silk–collagen sponge scaffolds *in vitro*. Implantation of allogeneic TDSC-seeded scaffolds in a rabbit model promoted the regeneration of tendon injuries, resulting in greater physiologic ECM structure and better biomechanical function without eliciting an immunologic reaction (56). Despite these inspiring findings, additional studies are required to determine an effective method of enhancing the regeneration process and to understand the long-term effect of transplanted TDSCs.

5.5. The source of TDSCS

TDSCs are useful for tissue engineering, but a key limitation in using TDSCs to repair damaged tendon is the availability of autologous tendon tissues. Removal of tendon sections for deriving TDSCs leads to formation of a secondary lesion at the donor site; however, allogeneic transplantation may be an ideal way for tendon tissue engineering. Previous studies have shown that bMSCs are MHC II-negative and are able to suppress lymphocyte proliferation *in vitro* (61). TDSCs also have negative expression of MHC II and do not stimulate, but in fact actively suppress, allogenous peripheral blood mononuclear cells (56). Moreover, allogeneic TDSC-seeded scaffolds not only elicit a negligible immune reaction, but also decrease lymphocyte infiltration induced by the xenogeneic scaffold. Thus, the TDSCs are non-immunogenic and immunosuppressive (56), and TDSCs can also be utilized for allogeneic transplantation and tissue engineering. Allogeneic TDSCs can be easily isolated from the discarded tendon tissue generated during tendon and ligament surgery, such as residual tendon graft tissue in anterior cruciate ligament reconstruction and total knee replacement, and an allogeneic TDSC bank can also be established for future clinical application.

6. CONCLUSIONS

In this review we have summarized the characteristics of TDSCs, as well as their advantages in tissue regeneration. Like bMSCs, TDSCs exhibit stem cell characteristics, including clonogenicity, self-renewal, and multi-lineage differentiation capacities. As is the case for other stem cells, no single marker can identify TDSCs; rather, a combination of factors must be used. The percentage of TDSCs in tendons is much greater than bMSCs in bone marrow, and TDSCs also proliferate faster.

Moreover, TDSCs are resident cells in tendons; when implanted into tendon defects, TDSCs are in an environment within which they are familiar and are more likely to survive and differentiate into the correct cell type. However, several questions still remain before TDSCs can be used clinically for augmenting tendon healing. Specifically, the amount of cells, the combination of growth factors and mechanical stimuli, and the ideal delivery vehicle need to be determined. In addition, the clinical trials and long-term follow-up must be performed as well. Thus, TDSCs exhibit great potential for tendon regeneration; however, strategies to promote the *in vitro* expansion of TDSCs, explore TDSC niche factors, and delivery scaffolds are essential to provide opportunities for regenerative therapies.

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Abbreviations: TDSCs: tendon-derived stem cells; bMSCs: bone marrow mesenchymal stem cells; ECM: extracellular matrix; $O_{2:}$ oxygen; TNMD: tenomodulin; PDT: population doubling time; TBJ: tendon–bone junction; Scx: scleraxis; IGF-I: insulin-like growth factor-I; FGF-2: fibroblast growth factor-2; PRP: platelet-rich plasma; PDGF: platelet-derived growth factor; ETM: engineered tendon matrix

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