Published in final edited form as:

J Orthop Res. 2015 June ; 33(6): 785–792. doi:10.1002/jor.22885.

Biology and mechano-response of tendon cells: progress overview and perspectives†

Hui B. Sun^{1,2,*}, Christoph Schaniel^{3,4,5}, Daniel J. Leong^{1,2}, and James H-C. Wang⁶

¹Department of Orthopaedic Surgery, Albert Einstein College of Medicine, Bronx, NY

²Department of Radiation Oncology, Albert Einstein College of Medicine, Bronx, NY

³Department of Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, New York, NY

⁴Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY

⁵Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY

⁶Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA

Abstract

In this review, we summarize the group discussions on Cell Biology & Mechanics from the 2014 ORS/ISMMS New Frontiers in Tendon Research Conference. The major discussion topics included: 1) the biology of tendon stem/progenitor cells (TSPCs) and the potential of stem cell-based tendon therapy using TSPCs and other types of stem cells, namely, embryonic and/or induced pluripotent stem cells (iPSCs), 2) the biological concept and potential impact of cellular senescence on tendon aging, tendon injury repair and the development of degenerative disease, and 3) the effects of tendon cells' mechano-response on tendon cell fate and metabolism. For each topic, a brief overview is presented which summarizes the major points discussed by the group participants. The focus of the discussions ranged from current research progress, challenges and opportunities, to future directions on these topics. In the preparation of this manuscript, authors consulted relevant references as a part of their efforts to present an accurate view on the topics discussed.

Keywords

TSPCs; senescence; iPSCs; mechanical loading; tendon degeneration

†This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/jor.22885]

*Corresponding Author: 1300 Morris Park Avenue, Golding 101 Bronx, NY 10461 USA herb.sun@einstein.yu.edu Tel: (718) 430-4291 Fax: (718) 430-3259.

Author Contributions: HS, CS, DL, JW wrote the manuscript. All authors have read and approved the final submitted manuscript.

Introduction

When tendons are injured, a healing process is initiated similar to that in other connective tissues. Following the breakdown and removal of damaged matrix and cells, usually with the aid of dedicated phagocytic cells (e.g. macrophages), new extracellular matrix (ECM) must be synthesized, deposited and populated with new cells in order to restore normal tissue function.¹ These repair processes must be coordinated, therefore the actions of the cells that carry them out must be correspondingly regulated. In tendons, the primary resident cells are tenocytes, differentiated cells responsible for the maintenance of tendon integrity, remodeling and repair.² However, recent studies have identified an additional cell population in tendons named tendon stem/progenitor cells (TSPCs).³⁻⁵ Like stem cells present in adult tissues, TSPCs are believed to be the source of newly differentiated tenocytes, responsible for maintaining adequate tenocyte numbers in the tissue throughout life and replenishing them after injury. When tendons exhibit increased susceptibility to damage (e.g. in aging), do not always heal well spontaneously following injury, and often fail to achieve full functional recovery after traditional surgical repair⁶⁻⁹, this raises the possibility that numerical and/or functional deficits exist in the resident populations of tenocytes and/or TSPCs.

Manipulation of TSPCs or other stem cell populations has considerable potential to remedy such defects, but at least two challenges remain. One is to understand the basis for damage susceptibility and poor healing, and the second is to devise effective approaches to stem cell-based therapies. Concerning the first, it is known that mechanical loading is one of the key factors that maintains the integrity of tendons.¹⁰ While underuse and overuse can lead to tendon degeneration, exercise at appropriate levels of mechanical loading has been shown to have beneficial effects such as enhancing the mechanical properties of tendons.¹¹ On the other hand, aging is known to decrease the functional competence of the human body. Degenerative changes, especially in connective tissues such as tendons occur during aging.^{12; 13} In addition, aging leads to tendon cell senescence,¹⁴ which may increase the susceptibility to tendon disorders such as degenerative tendinopathy. Interestingly, appropriate mechanical loading has also been shown to strengthen aging tendons.¹⁵ However, it is not clear how mechanical loading such as exercise affects tendon cellular fate and function (e.g., senescence). Recent studies have shed some light on this topic and have also begun to address the second challenge, suggesting a role for TSPCs in the repair of tendon injuries.^{16; 17} In addition, future advances in the applicability of pluripotent stem cells, such as embryonic stem cells and/or induced-pluripotent stem cells (iPSCs), could potentially change the landscape of tissue repair and regenerative medicine, providing new opportunities for tendon recovery after injury or in cases of degenerative diseases such as tendinopathy.

In this review, we summarize the group discussions on Cell Biology & Mechanics at the 2014 ORS/ISMMS New Frontiers in Tendon Research Conference. These discussions focused mainly on current research progress, challenges and opportunities, and future directions in the following topics: biology of TSPCs, the role of TSPCs in tendon repair and degenerative disease, and the potential use of stem cells including TSPCs and iPSCs, for tendon repair or regeneration after tendon injury or degenerative tendinopathy.

I. Tendon stem/progenitor cells (TSPCs) and induced pluripotent stem cells (iPSCs)

Stem cells offer a great potential to treat challenging diseases that evade traditional treatments. Since current treatments for tendon disorders such as tendinopathy are only palliative and do not cure the underlying cause, these disorders may benefit from the use of stem cell therapy with TSPCs and/or iPSCs.

The identification of TSPCs is one of the most important discoveries in the tendon research field. In 2007, Bi *et al.* reported a cell population with generally recognized stem cell characteristics such as clonogenicity, multipotency, and self-renewal capacity in both human and mouse tendons.³ These tendon specific stem cells were termed TSPCs,³ and are also known as tendon stem cells (TSCs)⁵ or tendon-derived stem cells (TDSCs).⁴ Recent studies have suggested that TSPCs play a role in the repair of tendinopathy and tendon injury.¹⁶⁻¹⁸ Similarly, mesenchymal stem cells (MSCs) obtained from tissues such as bone marrow and adipose tissue have been studied for their promising potential in tendon healing and repair.¹⁹⁻²³

A second stem cell type that can potentially be used to treat tendon disorders is the induced pluripotent stem cell (iPSC). These cells have revolutionized the concept of the stem cell, its differentiation and commitment.^{24; 25} Researchers showed that using transcription factors it was possible to reprogram adult cells, such as dermal fibroblasts, into pluripotent stem cells, which can differentiate into other needed stem/progenitor cells.²⁶

The group discussion on this topic raised interesting questions on the identity and niche of TSPCs, the role of TSPCs in tendon repair and degenerative disease, and the potential for stem cell-based approaches, including use of TSPCs, MSCs, and iPSCs, in tendon tissue regeneration and tendinopathy treatment. The discussion of these questions are detailed below.

What is the identity of TSPCs?

TSPCs were originally identified based on *in vitro* colony forming capacity and multi-lineage differentiation potential,³ and have been further demonstrated *in vitro* to express a panel of MSC associated surface markers and stem cell markers including stem cell antigen-1 (Sca-1), Oct-4, nucleostemin, SSEA-4, Nanog, and Sox-2.^{3; 5; 14; 27; 28} Compared to bone marrow-derived mesenchymal stem cells (BMSCs), TSPCs express high levels of Scleraxis (Scx), a tendon-enriched specific transcription factor, and tenomodulin (Tnmd), a marker of adult tenocytes.³ Morphologically, TSPCs possess smaller cell bodies and larger nuclei than ordinary tenocytes and have a cobblestone-like morphology in confluent cell cultures, whereas tenocytes are highly elongated, a typical phenotype of fibroblast-like cells.⁵ TSPCs also proliferate more quickly than tenocytes in culture,⁵ and when implanted *in vivo*, TSPCs exhibit the ability to regenerate tendon-like tissues.³

One of the challenges in characterizing TSPCs, especially *in vivo*, has been a lack of specific markers. Stem cells have been identified in the perivascular regions and in between parallel collagen fibrils in the tendon proper by immunostaining for classical stem cell markers, or

labeling cells with bromodeoxyuridine (BrdU) or iododeoxyuridine (IdU).^{3; 29; 30} Another study showed the presence of progenitor pools in the Achilles tendon-proper and paratenon, with both pools exhibiting distinct properties and containing enriched progenitor subpopulations of different origins.³¹

The population of TSPCs and their fate in the tendon tissue can vary, depending on the physiological or pathological status of the tissue. When a tendon is damaged, stem cells may migrate from sources outside the tendon, such as from the circulation,^{32; 33} skeletal muscles,³⁴ adipose tissue near tendons,³⁵ and the bursa, which is a fluid-filled sac that acts as a cushion between a bone and other moving parts such as muscles, tendons, or skin.^{36; 37} Based on these discussion topics the group agreed on the general obscurity in this area, and recommended further studies to consider focusing on the exact origins and identities of TSPCs (e.g. cell lineage markers and tracking) *in vivo*, and in particular, after a tendon injury.

What is the TSPC niche and what do we know about it?

Stem cell niches are dynamic microenvironments that interact with adult stem cells and regulate their fate such as quiescence, self-renewal and/or differentiation through cellular and non-cellular components.²⁹ Identification of functional niche components that maintain the ‘stemness’ of TSPCs and regulate their differentiation is important for three primary reasons: 1) to understand the TSPC biology *in vivo*, 2) to mobilize endogenous TSPCs in order to increase local TSPC levels, and 3) to produce *in vitro* sufficient quantities of TSPCs that mimic *in vivo* TSPC characteristics for potential therapeutic applications.

The TSPC niche is not well defined. Niche components that likely regulate TSPCs include the extracellular matrix, soluble factors, and the surrounding mechanical forces.²⁹ It has been reported that TSPCs reside within a unique niche, where two extracellular matrix proteins, biglycan and fibromodulin, regulate their function by modulating BMP and Wnt3a signaling.³ BMP-2 has been shown to promote non-tenocyte differentiation and proteoglycan deposition of TDSCs *in vitro*^{38; 39} while Wnt3a is known to promote the osteogenic differentiation of TDSCs *in vitro*.⁴⁰ Indeed, TSPCs isolated from the biglycan and fibromodulin double knock-out animal model displayed higher sensitivity to BMP-2 signaling.³ Besides, healing tendon cells in animal models and clinical samples of tendinopathy displayed an increase in the expression of chondroosteogenic BMPs and Wnt3a, chondrocyte-like cells and ossified deposits leading to “in-tendon ossification.”^{3; 41; 42} These findings provide the first insight into the role the molecular niche in the appropriate maintenance of TPSCs. The importance of tendon ECM in the maintenance of TSPC stemness was further supported by a recent study showing that rabbit TDSCs cultured on decellularized tendon matrix proliferated at a higher rate and had better stemness properties than TDSCs cultured on plastic tissue culture surface.⁴³

The multi-potency of TSPCs demands a molecular mechanism that prevents their erroneous differentiation into non-tenocytes. A previous study showed that an activated form of Smad8 protein inhibited the BMP-2-induced osteogenic differentiation of MSCs while promoting their tenogenic differentiation.⁴⁴ Similarly, Msh homeobox 2 (MSX2) also acted as a molecular defense to prevent ossification in ligament fibroblasts.⁴⁵

These findings on the TSPC niche are just the beginning of future studies. Such studies will reveal exciting new information on the interactions of TSPCs with surrounding matrix proteins, neighboring cells, mechanical and chemical signals, as well as the role of these interactions in tendon homeostasis, tendon's response to injury, such as TSPC migration from their home niche to the wounded site. The findings of future studies may lead to the development of novel interventions for tendon wound healing and disease treatment by improving the function of stem cells via a niche-based strategy.

What is the potential of a stem cell-based approach for tendon tissue regeneration and tendinopathy treatment?

Currently, there are no published clinical trials on the use of TSPCs to treat tendon injuries. However, previous studies have suggested that BMSCs may have therapeutic potential and may improve tendon healing. When BMSCs were introduced in the injured equine tendons, the re-injury rate was lower compared to tendons treated with conventional non-cellular based management.^{46; 47} Intratendinous injection of autologous BMSCs into a collagenase-induced tendinopathy model effectively induced tendon regeneration.⁴⁸ Targeted intralesional injection of BMSCs into equine superficial digital flexor with tendinopathy resulted in the recovery of normal activity in horses.⁴⁹ Also, adipose derived stem cells were shown to effectively treat equine tendinopathies leading to complete recovery and return to normal activity in horses.⁵⁰ Together, these preclinical studies suggest that stem cells, and more likely TSPCs, hold promise for the treatment of tendon disorders. However, as a cautionary note, implantation of BMSCs into injured tendons has been reported to induce ectopic bone formation in a rabbit tendon wound model,⁵¹ suggesting that adult stem cells from a specific tissue may have a tendency to differentiate into undesired cells of their tissue of origin.

The group agreed that a better understanding on the basics of TSPC biology is critical for its potential use in tendon tissue repair and disease treatment. In particular, since TSPCs are a heterogeneous population, their precise isolation from tendon tissues is critical for effective treatment purposes. However, overemphasis on the search for TSPC-specific molecular markers may not advance efforts to obtain sufficient high quality TSPCs for clinical use.

II. Cellular senescence and its biological impact on tendon tissues

Cell senescence refers to the cell status in which permanent cell cycle arrest cannot be reactivated.⁵² Recent studies have shown that senescent cells are live cells, but with altered functions. For example, they secrete MMPs, resist apoptosis, and express senescence markers such as β -galactosidase (β -gal).⁵² Cell senescence is prevalent in aged tendon tissues,^{53; 54} although it is not clear whether and how cell senescence also occurs in young tendons, or in response to oxidative, inflammatory or overloading stresses. This topic initiated an enthusiastic group discussion, especially with reference to the recently re-established concepts of cell fate determination and reprogramming.

Does cell senescence occur in young tendon cells?

Although cell senescence occurs during the aging process,⁵⁵ stress and disease conditions may facilitate premature senescence in young cells. Senescent cells are characterized by the elevated expression of senescent cell markers such as β -gal, senescence-associated genes such as p53, p21, and p16^{INK4a}, MMPs, ADAMTS, and pro-inflammatory cytokines. They exhibit improper proliferation and impaired survival due to compromised differentiation and maturation.⁵⁶ Based on these findings, the group agreed that the concept of cell senescence is no longer limited to aged cells but may also extend to young cells under specific circumstances. Therefore, cell senescence could be a special fate and functional status of a cell that critically impacts tendon tissue homeostasis, tissue degeneration, injury, and repair and regeneration.

What do we know about the molecular regulation of TSPC aging and senescence?

Currently, there is no substantial published work on senescence of tendon cellular components and its relationship to tendinopathy. The limited studies have shown that aging changes the self-renewal and differentiation capability of TSPCs. The following findings have been reported on TSPCs isolated from aged animals: numbers of colony-forming TSPCs were lower, proliferation decreased, cell cycle progression was delayed, cell fate patterns were altered, expression of tendon lineage markers decreased, adipocytic differentiation increased, expression of CITED2, a multi-stimuli responsive transactivator involved in cell growth and senescence, was downregulated, and CD44, a matrix assembling and organizing protein implicated in tendon healing, was upregulated.¹⁴ These findings highlight the detrimental effects of aging on TSPCs.

Tenomodulin (Tnmd) has been demonstrated to have a specific role in maintaining homeostasis of TSPCs. Loss of Tnmd, as shown in Tnmd knockout (KO) mice, resulted in reduced self-renewal and augmented senescence of TSPCs.⁵⁷ This was also reflected in lowered Cyclin D1 levels. Normal proliferation was restored by transient transfection of Tnmd cDNA into Tnmd KO TSPCs. In addition, self-renewal capacity of Tnmd KO TSPCs was lower and the expression of the cell cycle inhibitor, p53, was higher when compared to control TSPCs. Interestingly, Tnmd KO did not significantly affect the multi-differentiation potential of aging TSPCs.⁵⁷

miR-135a has also been suggested to regulate TSPC senescence. Expression of miR-135a is suppressed in aged TSPCs, and miR-135a overexpression suppressed senescence and promoted the proliferation and tenogenic differentiation of TSPCs. These effects of miR-135a were shown to be mediated by Rho-associated coiled-coil protein kinase 1 (ROCK1).⁵⁸

The group agreed that further studies on the mechanisms underlying the regulation of TSPCs by aging will propel this area of research in a new direction. The new findings could provide clues to understand TSPC survival, stress response, function and fate determination, which are critical for the intervention of injuries and diseases, especially those related to aging.

Is there a link between cell senescence and tendinopathy?

Tendinopathies are common musculoskeletal injuries that lead to pain and disability. The development of tendinopathy is attributed to progressive pathological changes in tendons that consequently compromise tendon structure and function.⁵⁹ Results from a recent study suggests that in tendinopathy, exogenous inflammatory cytokines are released from immigrated leukocytes, which trigger tenocytes to produce pro-inflammatory mediators, and proteases such as MMPs.⁶⁰ One of the characteristics of senescent cells is an excessive increase in the levels of MMPs, ADAMTS, and pro-inflammatory cytokines such as TNF- α and IL-1 β .⁵² Furthermore, although tendinopathy and cell senescence are associated with aging, both may occur at a young age. A recent study demonstrated that TSPCs from a collagenase-induced tendon injury model exhibited higher cellular senescence, which was associated with a lower proliferative capacity and failed tendon healing.⁶¹ Taken together, these findings suggest a critical link between TSPC senescence and tendinopathies. Future studies on the role of aging in tenocytes and TSPCs will provide insights into the etiology and development of tendinopathies and may lead to novel prevention and treatment strategies by intervening in cell fate pathways that regulate cellular senescence.

III. Mechano-response of tendon cells

An essential role of tendons is to transfer tensile loads from muscle to bone to enable joint motions. Therefore, tendon cells are constantly subjected to mechanical loads.¹¹ In this regard, mechanical loading is one of the most important factors for tendon homeostasis and a critical factor for the function and fate determination of tenocytes and TSPCs.¹⁰ On the other hand, tendons adaptively change their structure and function in response to mechanical loading, which largely relies on the mechano-responsibility of resident tendon cells.¹¹ It has been well established that tenocytes, the dominant tendon cell type, respond to mechanical loading in a loading intensity dependent manner. Mechanical loading at physiological levels is essential for normal functioning of tenocytes and is associated with healthy tendons, while excessive loading alters the function of tenocytes, which is detrimental to tendons¹¹. This principle is also largely applicable to recently discovered TSPCs.^{62; 63}

How does mechanical loading affect cell fate and function of tendons?

Mechanical loading is critical for the maintenance of tendon homeostasis. Studies have shown clearly that mechanical loading is one of the determining factors of tendon cell functions. Mechanical loading at physiological conditions (i.e., 4% cyclic uniaxial stretch *in vitro*) has been shown to balance the metabolism of tenocytes by enhancing anabolic activity such as production of type I collagen, and reducing catabolic activities such as suppressing IL-1 elevated cyclooxygenase-2 (COX-2) and MMP-1 gene expression and PGE₂ production.⁶⁴ In contrast, large magnitudes of mechanical loading (i.e., 8% cyclic uniaxial stretch) can shift the metabolic balance toward catabolism by increasing the levels of the inflammatory mediators, COX-2 and PGE₂.⁶⁴ The mechanical loading-induced altered cellular metabolism in tenocytes may be responsible for the induction of neutrophil infiltration, tissue edema, and reduction in tenocyte proliferation and type I collagen synthesis, which are pathological changes observed in tendinopathic tendons.⁶⁴

Studies on TSPCs support the notion that mechanical loading is a critical factor in tendon stem cell fate. An *in vitro* study showed that mechanical loading at physiological levels promoted TSPC proliferation and differentiation into tenocytes, while excessive levels of loading led TSPCs to differentiate into non-tenocytes such as adipocytes, chondrocytes and osteocytes, in addition to tenocytes.⁶³ An *in vivo* study using treadmill running further found that tendons subjected to repetitive strenuous mechanical loading produced high levels of PGE₂, which was associated with decreased TSPC proliferation and induced TSPCs to differentiate into adipocytes and osteocytes.⁶⁵ These studies suggest that non-physiological loading may induce tendinopathy, at least in part, by altering TSPC function and fate at both the proliferation and differentiation levels. Better understanding of these mechanisms may provide a new strategy for the prevention and treatment of tendinopathy.

Can mechanical loading (e.g. through exercise) “wake up” senescence cells in tendons? If so, by what mechanism?

As described above, senescent cells are live cells with altered function such as production of excessive levels of MMPs, ADAMTS, and pro-inflammatory cytokines.⁵⁶ They also have an impaired regeneration and repair capacity in response to age-related stress such as oxidative stress, non-physiological loading and cytokine exposure. Studies in tenocytes and chondrocytes have suggested that physiological loading may reduce the production of MMPs, ADAMTS, pro-inflammatory cytokines and mediators, and may reduce the production of oxidative products such as ROS.^{66; 67} It was found that mechanical loading increased the number of TSPCs in both patellar and Achilles tendons in mice subjected to treadmill running.⁶⁸ Although a direct evidence for the influence of mechanical loading on senescent cells is lacking, these previous studies suggest that mechanical loading increases TSPC numbers, in part, by “awakening” or reactivating senescent cells from their cell cycle arrest. These studies have just begun exploring the plasticity of senescent cells. The group discussion concluded that physiological loading may be beneficial in slowing cellular aging and improving aging-associated impaired healing ability by reactivating senescent tendon cells, especially TSPCs. Therefore this topic warrants future study.

IV. Induced pluripotent stem cells (iPSCs) and their applicability for tendon repair and regeneration

Induced pluripotent stem cells (iPSCs) were originally generated using viral vectors to introduce key reprogramming factors (Oct-3/4 and Sox-2, with KLF4 and C-MYC or NANOG and LIN28) into skin fibroblasts of mice then humans, or into other terminally differentiated cells obtained from patients.^{24; 25; 69} These reprogramming factors induced an embryonic-like state in adult cells, which can be passaged in culture indefinitely while retaining their pluripotency. These cells can be also differentiated via specific signaling cues into cell types derived from any of the three germ layers (endoderm, mesoderm, and ectoderm). The first generation of iPSCs was created using DNA-integrating viruses, but since this method introduces genomic alterations, current research protocols achieve reprogramming using non-integrative vectors such as Sendai virus, non-integrating adeno-associated virus, episomal vectors, microRNAs, mRNA transfection, or PiggyBac transposons to minimize the genomic perturbations.⁷⁰⁻⁷⁴ These revolutionary achievements

are changing the field of stem cell research and regenerative medicine that will impact the treatment of tendon disorders.

iPSCs in the tendon field: what has been done?

To our excitement, it was reported that human iPSC-derived neural crest stem cells (NCSCs) promoted tendon repair in a rat patellar tendon window defect model.⁷⁵ iPSC-NCSCs were suspended in fibrin gel and transplanted into a rat patellar tendon window defect. At 4 weeks post-transplantation, macroscopical observation showed that the repair of iPSC-NCSC-treated tendons was superior to the non-iPSC-NCSC-treated tendons. Histological and mechanical analyses revealed that iPSC-NCSCs treatment significantly enhanced tendon healing as indicated by the improvement in matrix synthesis and mechanical properties. Furthermore, transplanted iPSC-NCSCs produced fetal tendon-related matrix proteins, stem cell recruitment factors, and tenogenic differentiation factors, and accelerated the host endogenous repair process.⁷⁵ This study demonstrates a potential strategy to employ iPSC-derived NCSCs for tendon tissue engineering. The study also shows the first evidence to support the use of iPSCs to enhance tendon wound healing and repair.

What is our perspective on using iPSCs for disease treatment and disease modeling?

Although the use of murine models for human diseases is standard in biomedical research, animal models may not accurately mimic human diseases.^{76; 77} Therefore, conventional mouse models, along with non-human *in vitro* and *in vivo* systems, have inherent limitations in representing human disease pathology and in testing potential therapeutics. Since the description of iPSC technology in 2006 (mouse) and 2007 (human), about 70 human iPSC (hiPSC) models of human diseases have been published.⁷⁸ Patient-specific hiPSCs can be produced to reproduce aspects of the disease phenotype *in vitro* and may also reveal underlying characteristics that are not clinically evident.⁷⁹

As tendinopathy is largely due to the altered fate and functional status of cells from the tendon-lineage, the group agreed that iPSCs could be a promising autologous source for tendon wound repair and regeneration. However, considering the unmet challenge of differentiating sufficient numbers of hiPSCs into high quality tendon stem cells for repair and regeneration, modeling tendon diseases such as tendinopathy using hiPSCs might provide a more realistic opportunity. Such studies may help uncover the mechanisms underlying such diseases and could establish the basis for drug screening or other interventions.

V. Closing remarks

Cells in tendon tissues maintain the ECM and play a central role in tendon homeostasis, wound repair and, when altered, lead to the failure of tissue repair and disease conditions such as tendinopathy. Since the discovery of TSPCs in 2007, TSPCs have drawn great attention and represent a great potential strategy for tendon repair or regeneration. TSPCs may also play an important role in the development of degenerative tendinopathy by undergoing aberrant differentiation into non-tenocyte lineages of cells in response to excessive mechanical loading placed on the tendons.⁶²

Further, the discovery of iPSCs and cell fate changes via reprogramming provide great possibilities for wound repair and regenerative medicine. Current understanding of the high plasticity of connective tissue cells, including tendon cells, are changing the basic concepts in cell biology, particularly concerning cell senescence and cell fate determination. These insights may provide great opportunities for using non-invasive, non-toxic means such as mechanical loading to modify the altered fate and function of cells in tendon, particularly stem cells. The group agreed that although steady progress has been made, research in tendon cell biology and mechanobiology is still far behind other musculoskeletal fields such as bone and cartilage research. However, the topics discussed here reveal unique and tremendous opportunities that may contribute to a better basic understanding of tendon cells and provide strategies and solutions for tendon aging, wound repair and regeneration.

Acknowledgements

The authors would like to thank the group discussion participants for their contributions to this work and Dr. Robert Majeska for critically reading this manuscript. The authors wish to acknowledge the funding support from NIH AG039561 (HBS), AR061395 and AR060920 (JHW).

References

1. Sharma P, Maffulli N. Tendon injury and tendinopathy: healing and repair. *J Bone Joint Surg Am*. 2005; 87:187–202. [PubMed: 15634833]
2. Sharir A, Zelzer E. Tendon homeostasis: the right pull. *Curr Biol*. 2011; 21:R472–474. [PubMed: 21683902]
3. Bi Y, Ehrichtou D, Kilts TM, et al. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nat Med*. 2007; 13:1219–1227. [PubMed: 17828274]
4. Rui YF, Lui PP, Li G, et al. Isolation and characterization of multipotent rat tendon-derived stem cells. *Tissue Eng Part A*. 2010; 16:1549–1558. [PubMed: 20001227]
5. Zhang J, Wang JH. Characterization of differential properties of rabbit tendon stem cells and tenocytes. *BMC Musculoskelet Disord*. 2010; 11:10. [PubMed: 20082706]
6. Lorbach O, Tompkins M. Rotator cuff: biology and current arthroscopic techniques. *Knee Surg Sports Traumatol Arthrosc*. 2012; 20:1003–1011. [PubMed: 22270674]
7. Galatz LM, Ball CM, Teefey SA, et al. The outcome and repair integrity of completely arthroscopically repaired large and massive rotator cuff tears. *J Bone Joint Surg Am*. 2004; 86-A: 219–224. [PubMed: 14960664]
8. Jones MP, Khan RJ, Carey Smith RL. Surgical interventions for treating acute achilles tendon rupture: key findings from a recent cochrane review. *J Bone Joint Surg Am*. 2012; 94:e88. [PubMed: 22717840]
9. Dy CJ, Hernandez-Soria A, Ma Y, et al. Complications after flexor tendon repair: a systematic review and meta-analysis. *The Journal of hand surgery*. 2012; 37:543–551. e541. [PubMed: 22317947]
10. Wang JH, Guo Q, Li B. Tendon biomechanics and mechanobiology--a minireview of basic concepts and recent advancements. *J Hand Ther*. 2012; 25:133–140. quiz 141. [PubMed: 21925835]
11. Wang JH. Mechanobiology of tendon. *J Biomech*. 2006; 39:1563–1582. [PubMed: 16000201]
12. Magnusson SP, Beyer N, Abrahamsen H, et al. Increased cross-sectional area and reduced tensile stress of the Achilles tendon in elderly compared with young women. *J Gerontol A Biol Sci Med Sci*. 2003; 58:123–127. [PubMed: 12586849]
13. Coupe C, Hansen P, Kongsgaard M, et al. Mechanical properties and collagen cross-linking of the patellar tendon in old and young men. *J Appl Physiol*. 2009; 107:880–886. [PubMed: 19556458]
14. Zhou Z, Akinbiyi T, Xu L, et al. Tendon-derived stem/progenitor cell aging: defective self-renewal and altered fate. *Aging Cell*. 2010; 9:911–915. [PubMed: 20569237]

15. LaCroix AS, Duenwald-Kuehl SE, Brickson S, et al. Effect of age and exercise on the viscoelastic properties of rat tail tendon. *Ann Biomed Eng.* 2013; 41:1120–1128. [PubMed: 23549897]
16. Shen W, Chen J, Yin Z, et al. Allogeneous tendon stem/progenitor cells in silk scaffold for functional shoulder repair. *Cell transplantation.* 2012; 21:943–958. [PubMed: 22405331]
17. Chen L, Dong SW, Liu JP, et al. Synergy of tendon stem cells and platelet-rich plasma in tendon healing. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society.* 2012; 30:991–997. [PubMed: 22161871]
18. Ni M, Lui PP, Rui YF, et al. Tendon-derived stem cells (TDSCs) promote tendon repair in a rat patellar tendon window defect model. *J Orthop Res.* 2012; 30:613–619. [PubMed: 21928428]
19. Bashir J, Sherman A, Lee H, et al. Mesenchymal stem cell therapies in the treatment of musculoskeletal diseases. *PM R.* 2014; 6:61–69. [PubMed: 24439148]
20. Lee JY, Zhou Z, Taub PJ, et al. BMP-12 treatment of adult mesenchymal stem cells in vitro augments tendon-like tissue formation and defect repair in vivo. *PLoS One.* 2011; 6:e17531. [PubMed: 21412429]
21. Chen X, Zou XH, Yin GL, et al. Tendon tissue engineering with mesenchymal stem cells and biografts: an option for large tendon defects? *Frontiers in bioscience.* 2009; 1:23–32.
22. Gulotta LV, Kovacevic D, Packer JD, et al. Bone marrow-derived mesenchymal stem cells transduced with scleraxis improve rotator cuff healing in a rat model. *Am J Sports Med.* 2011; 39:1282–1289. [PubMed: 21335341]
23. Gulotta LV, Kovacevic D, Ehteshami JR, et al. Application of bone marrow-derived mesenchymal stem cells in a rotator cuff repair model. *Am J Sports Med.* 2009; 37:2126–2133. [PubMed: 19684297]
24. Park IH, Zhao R, West JA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature.* 2008; 451:141–146. [PubMed: 18157115]
25. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007; 131:861–872. [PubMed: 18035408]
26. Fox IJ, Daley GQ, Goldman SA, et al. Stem cell therapy. Use of differentiated pluripotent stem cells as replacement therapy for treating disease. *Science.* 2014; 345:1247391.
27. Tan Q, Lui PP, Lee YW. In vivo identity of tendon stem cells and the roles of stem cells in tendon healing. *Stem Cells Dev.* 2013; 22:3128–3140. [PubMed: 23815595]
28. Lovati AB, Corradetti B, Lange Consiglio A, et al. Characterization and differentiation of equine tendon-derived progenitor cells. *J Biol Regul Homeost Agents.* 2011; 25:S75–84. [PubMed: 22051173]
29. Lui PP. Identity of tendon stem cells--how much do we know? *J Cell Mol Med.* 2013; 17:55–64. [PubMed: 23279609]
30. Tempfer H, Wagner A, Gehwolf R, et al. Perivascular cells of the supraspinatus tendon express both tendon- and stem cell-related markers. *Histochem Cell Biol.* 2009; 131:733–741. [PubMed: 19280209]
31. Mienaltowski MJ, Adams SM, Birk DE. Regional differences in stem cell/progenitor cell populations from the mouse achilles tendon. *Tissue Eng Part A.* 2013; 19:199–210. [PubMed: 22871316]
32. Woo SL, Hildebrand K, Watanabe N, et al. Tissue engineering of ligament and tendon healing. *Clin Orthop Relat Res.* 1999:S312–323. [PubMed: 10546655]
33. Kajikawa Y, Morihara T, Watanabe N, et al. GFP chimeric models exhibited a biphasic pattern of mesenchymal cell invasion in tendon healing. *J Cell Physiol.* 2007; 210:684–691. [PubMed: 17154365]
34. Peault B, Rudnicki M, Torrente Y, et al. Stem and progenitor cells in skeletal muscle development, maintenance, and therapy. *Mol Ther.* 2007; 15:867–877. [PubMed: 17387336]
35. Buckley CT, Vinardell T, Thorpe SD, et al. Functional properties of cartilaginous tissues engineered from infrapatellar fat pad-derived mesenchymal stem cells. *J Biomech.* 2010; 43:920–926. [PubMed: 20005518]
36. Utsunomiya H, Uchida S, Sekiya I, et al. Isolation and characterization of human mesenchymal stem cells derived from shoulder tissues involved in rotator cuff tears. *Am J Sports Med.* 2013; 41:657–668. [PubMed: 23371475]

37. Song N, Armstrong AD, Li F, et al. Multipotent mesenchymal stem cells from human subacromial bursa: potential for cell based tendon tissue engineering. *Tissue Eng Part A*. 2014; 20:239–249. [PubMed: 23865619]
38. Rui YF, Lui PP, Ni M, et al. Mechanical loading increased BMP-2 expression which promoted osteogenic differentiation of tendon-derived stem cells. *J Orthop Res*. 2011; 29:390–396. [PubMed: 20882582]
39. Rui YF, Lui PP, Wong YM, et al. BMP-2 stimulated non-tenogenic differentiation and promoted proteoglycan deposition of tendon-derived stem cells (TDSCs) in vitro. *J Orthop Res*. 2013; 31:746–753. [PubMed: 23238867]
40. Lui PP, Lee YW, Wong YM, et al. Expression of Wnt pathway mediators in metaplastic tissue in animal model and clinical samples of tendinopathy. *Rheumatology (Oxford)*. 2013; 52:1609–1618. [PubMed: 23776285]
41. Rui YF, Lui PP, Rolf CG, et al. Expression of chondro-osteogenic BMPs in clinical samples of patellar tendinopathy. *Knee Surg Sports Traumatol Arthrosc*. 2012; 20:1409–1417. [PubMed: 21946950]
42. Yee Lui PP, Wong YM, Rui YF, et al. Expression of chondro-osteogenic BMPs in ossified failed tendon healing model of tendinopathy. *J Orthop Res*. 2011; 29:816–821. [PubMed: 21520255]
43. Zhang J, Li B, Wang JH. The role of engineered tendon matrix in the stemness of tendon stem cells in vitro and the promotion of tendon-like tissue formation in vivo. *Biomaterials*. 2011; 32:6972–6981. [PubMed: 21703682]
44. Hoffmann A, Pelled G, Turgeman G, et al. Neotendon formation induced by manipulation of the Smad8 signalling pathway in mesenchymal stem cells. *J Clin Invest*. 2006; 116:940–952. [PubMed: 16585960]
45. Yoshizawa T, Takizawa F, Iizawa F, et al. Homeobox protein MSX2 acts as a molecular defense mechanism for preventing ossification in ligament fibroblasts. *Mol Cell Biol*. 2004; 24:3460–3472. [PubMed: 15060165]
46. Smith RK. Mesenchymal stem cell therapy for equine tendinopathy. *Disabil Rehabil*. 2008; 30:1752–1758. [PubMed: 18608378]
47. Godwin EE, Young NJ, Dudhia J, et al. Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon. *Equine Vet J*. 2012; 44:25–32. [PubMed: 21615465]
48. Lacitignola L, Crovace A, Rossi G, et al. Cell therapy for tendinitis, experimental and clinical report. *Vet Res Commun*. 2008; 32(Suppl 1):S33–38. [PubMed: 18686004]
49. Pacini S, Spinabella S, Trombi L, et al. Suspension of bone marrow-derived undifferentiated mesenchymal stromal cells for repair of superficial digital flexor tendon in race horses. *Tissue Eng*. 2007; 13:2949–2955. [PubMed: 17919069]
50. Del Bue M, Ricco S, Ramoni R, et al. Equine adipose-tissue derived mesenchymal stem cells and platelet concentrates: their association in vitro and in vivo. *Vet Res Commun*. 2008; 32(Suppl 1):S51–55. [PubMed: 18683070]
51. Awad HA, Boivin GP, Dressler MR, et al. Repair of patellar tendon injuries using a cell-collagen composite. *J Orthop Res*. 2003; 21:420–431. [PubMed: 12706014]
52. Campisi J, d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. *Nat Rev Mol Cell Biol*. 2007; 8:729–740. [PubMed: 17667954]
53. Chang E, Harley CB. Telomere length and replicative aging in human vascular tissues. *Proc Natl Acad Sci U S A*. 1995; 92:11190–11194. [PubMed: 7479963]
54. Price JS, Waters JG, Darrah C, et al. The role of chondrocyte senescence in osteoarthritis. *Aging Cell*. 2002; 1:57–65. [PubMed: 12882354]
55. van Deursen JM. The role of senescent cells in ageing. *Nature*. 2014; 509:439–446. [PubMed: 24848057]
56. Loeser RF. Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix. *Osteoarthritis Cartilage*. 2009; 17:971–979. [PubMed: 19303469]
57. Alberton P, Dex S, Popov C, et al. Loss of tenomodulin results in reduced self-renewal and augmented senescence of tendon stem/progenitor cells. *Stem Cells Dev*. 2014

58. Chen L, Wang GD, Liu JP, et al. miR-135a modulates tendon stem/progenitor cell senescence via suppressing ROCK1. *Bone*. 2015; 71:210–216. [PubMed: 25460182]
59. Goldin M, Malanga GA. Tendinopathy: a review of the pathophysiology and evidence for treatment. *The Physician and sportsmedicine*. 2013; 41:36–49. [PubMed: 24113701]
60. Schulze-Tanzil G, Al-Sadi O, Wiegand E, et al. The role of pro-inflammatory and immunoregulatory cytokines in tendon healing and rupture: new insights. *Scandinavian journal of medicine & science in sports*. 2011; 21:337–351. [PubMed: 21210861]
61. Rui YF, Lui PP, Wong YM, et al. Altered fate of tendon-derived stem cells isolated from a failed tendon-healing animal model of tendinopathy. *Stem Cells Dev*. 2013; 22:1076–1085. [PubMed: 23106341]
62. Zhang J, Wang JH. The effects of mechanical loading on tendons--an in vivo and in vitro model study. *PLoS One*. 2013; 8:e71740. [PubMed: 23977130]
63. Zhang J, Wang JH. Mechanobiological response of tendon stem cells: implications of tendon homeostasis and pathogenesis of tendinopathy. *J Orthop Res*. 2010; 28:639–643. [PubMed: 19918904]
64. Yang G, Im HJ, Wang JH. Repetitive mechanical stretching modulates IL-1beta induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts. *Gene*. 2005; 363:166–172. [PubMed: 16226404]
65. Zhang J, Wang JH. Production of PGE(2) increases in tendons subjected to repetitive mechanical loading and induces differentiation of tendon stem cells into non tenocytes. *J Orthop Res*. 2010; 28:198–203. [PubMed: 19688869]
66. Sun HB. Mechanical loading, cartilage degradation, and arthritis. *Ann N Y Acad Sci*. 2010; 1211:37–50. [PubMed: 21062294]
67. Sun HB, Li Y, Fung DT, et al. Coordinate regulation of IL-1beta and MMP-13 in rat tendons following subrupture fatigue damage. *Clin Orthop Relat Res*. 2008; 466:1555–1561. [PubMed: 18470577]
68. Zhang J, Pan T, Liu Y, et al. Mouse treadmill running enhances tendons by expanding the pool of tendon stem cells (TSCs) and TSC-related cellular production of collagen. *J Orthop Res*. 2010; 28:1178–1183. [PubMed: 20225313]
69. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
70. Okita K, Yamakawa T, Matsumura Y, et al. An efficient nonviral method to generate integration-free human-induced pluripotent stem cells from cord blood and peripheral blood cells. *Stem Cells*. 2013; 31:458–466. [PubMed: 23193063]
71. Kaji K, Norrby K, Paca A, et al. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature*. 2009; 458:771–775. [PubMed: 19252477]
72. Miyoshi N, Ishii H, Nagano H, et al. Reprogramming of mouse and human cells to pluripotency using mature microRNAs. *Cell stem cell*. 2011; 8:633–638. [PubMed: 21620789]
73. Anokye-Danso F, Trivedi CM, Juhr D, et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. *Cell stem cell*. 2011; 8:376–388. [PubMed: 21474102]
74. Warren L, Manos PD, Ahfeldt T, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell stem cell*. 2010; 7:618–630. [PubMed: 20888316]
75. Xu W, Wang Y, Liu E, et al. Human iPSC-derived neural crest stem cells promote tendon repair in a rat patellar tendon window defect model. *Tissue Eng Part A*. 2013; 19:2439–2451. [PubMed: 23815150]
76. Seok J, Warren HS, Cuenca AG, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:3507–3512. [PubMed: 23401516]
77. Burkhardt AM, Zlotnik A. Translating translational research: mouse models of human disease. *Cellular & molecular immunology*. 2013; 10:373–374. [PubMed: 23934028]
78. Ko HC, Gelb BD. Concise review: drug discovery in the age of the induced pluripotent stem cell. *Stem Cells Transl Med*. 2014; 3:500–509. [PubMed: 24493856]

79. Colman A, Dreesen O. Pluripotent stem cells and disease modeling. *Cell stem cell*. 2009; 5:244–247. [PubMed: 19733533]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript