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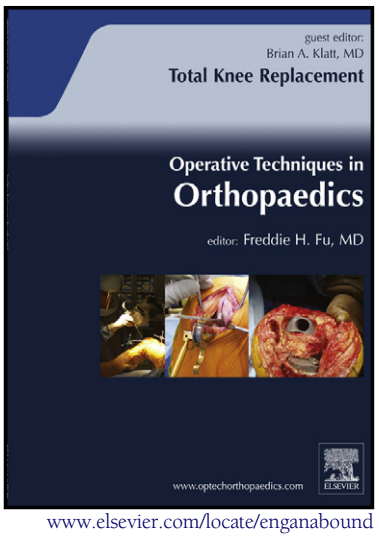

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James H-C. Wang, Xavier Nirmala

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Application of TSCs and PRP to Treat Tendon Injuries

[#]James H-C. Wang, and Xavier Nirmala

MechanoBiology Laboratory, Department of Orthopaedic Surgery,
University of Pittsburgh School of Medicine, Pittsburgh, USA

#Correspondence to:
James H-C. Wang, PhD
210 Lothrop Street, BST, E1640, Pittsburgh, PA 15213
Tel.: 412-648-9102; Fax: 412-648-8548
wanghc@pitt.edu

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Abstract

Tendon injuries like tendinopathy are a serious healthcare problem in the United States. However, current treatments for tendon injuries are largely palliative. Biologics treatments, including tendon stem/progenitor cells (TSCs) and platelet rich plasma (PRP) hold great potential to effectively treat tendon injuries. TSCs are tendon specific stem cells and have the ability to differentiate into tenocytes, the resident tendon cells responsible for tendon homeostasis and tendon repair in case of an injury. TSCs can also self-renew and thus can replenish the tendon with tendon cells (TSCs and tenocytes) to maintain a healthy tendon. The action of PRP can be complementary; PRP can augment and accelerate tendon healing by supplying abundant growth factors contained in platelets, and fibrin matrix, which functions as a natural conducive scaffold to facilitate tissue healing. This article provides a summary of the

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findings in recent basic and clinical studies on the applications of TSCs and PRP to the treatment of tendon injuries. It also outlines the challenges facing their applications in clinical settings. In particular, the controversy surrounding the efficacy of PRP treatment for tendon injuries are analyzed and solutions are suggested.

Abbreviations

TSCs – Tendon stem cell/progenitor cells; PRP – Platelet Rich Plasma; MSCs – Mesenchymal Stem Cells; ASCs – Adipose-derived Stem Cells; IGF-1 – Insulin-like Growth Factor-1; VEGF – Vascular Endothelial Growth Factor; TGF-β – Transforming Growth Factor β; HGF – Hepatocyte Growth Factor; PDGF – Platelet Derived Growth Factor; EGF – Epidermal Growth Factor; FGF – Fibroblastic Growth Factor; BDSCs – Blood Derived Stem Cells.

Introduction

Tendon injuries are highly prevalent in athletic settings with an estimated 40-50% of athletes getting tendon injuries (1-3). However, tendon injuries are also common in occupational settings and in the aged population. Among the work-related injuries reported in hospitals in the Olmsted County, Minnesota, United States from 2001-2010 about 25% accounted for acute tendon injuries of the hands and wrists alone (4). In the aging population ~15% aged 50-59 years and ~51% aged 80 years and above are estimated to experience tendon injuries (5).

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Tendons are fibrous connective tissues containing 65-80% collagen type I, and elastin, proteoglycans, glycoproteins, and water in smaller amounts within tendon cells (6, 7). Since tendons link bones to muscles, they are designed to withstand mechanical loads, namely, the muscular loads. However, excessive or repetitive loads have a bearing on tendons and cause tendon injuries (2, 8-10). When exposed to these abnormal loading conditions, cellular activities in tendons are modified resulting in structural changes that finally compromise tendon function. In general two types of tendon injuries have been recognized: acute and chronic injuries. Acute injuries are tendon damages caused by mechanical over-loads on the tendon. Chronic injuries, often called tendinopathies, are mainly degenerative changes in the tendon caused, at least in sport settings, by mechanical over-use of the tendon.

After a tendon injury, natural healing takes place. However, the tendon healing is a slow and inefficient process, which does not restore the normal biological and biomechanical properties to injured tendons. Consequently, patients more often are unable to return their normal activities to pre-injury levels (11, 12). More importantly, the repaired region especially in returning athletes is at a higher risk for re-injury (13).

Despite its prevalence there is no consensus on the treatment method and management of tendon injuries. Most interventions are conventional and are limited to treating only the pain and inflammation symptoms using non-steroidal anti-inflammatory drugs (NSAIDs) (14, 15), cryotherapy (16, 17), physiotherapy (11, 18), etc. Thus, there is a pressing need for better treatment options to restore the normal tendon structure and function of an injured tendon.

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In recent years, the application of biological treatments or tissue engineering approaches is being eagerly sought for the treatment of tendon injuries. Among them, the use of stem cells particularly tendon stem/progenitor cells (TSCs) and platelet-rich-plasma (PRP) may have the most potential to improve the healing of injured tendons. Many studies have indicated that these two biologics treatments can augment the healing of tendon injuries. This review briefs the findings from these studies and provides discussion on the use of these biologics treatments to effectively repair injured tendons and subsequently improve tendon structure and function thus enabling patients to return quickly to work and sporting activities.

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Use of TSCs to treat tendon injuries

Until recently a common misconception about tendons was that they are made of one kind of cells namely tenocytes. However, recent studies have shown that about 5% of the tendon cells are TSCs, which are tendon-specific stem cells present in the tendons of mice, rabbits, rats and humans (19-22). In several characteristics, TSCs differ from tenocytes, which are dominant residential cells in tendons. These include: 1) *Morphology*: TSCs in culture are more cobble-stone shaped with larger nuclei while the tenocytes are more elongated and have smaller nuclei (20); 2) *Proliferation*: TSCs grow faster than tenocytes *in vitro;* 3) *Stemness*: TSCs in culture express stem cell markers, Oct-4, SSEA-1 & 4 and nucleostemin (NS), which are not expressed by tenocytes (9, 20); and 4) *Multi-differentiation*: TSCs have the ability to differentiate into tenocytes as well as into several non-tendon cell types including adipocytes, chondrocytes and osteocytes (19, 20).

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Recent years have seen an increased interest in the use of adult stem cells in biologics treatments for tendon injuries. For example, mesenchymal stem cells (MSCs) have been used to treat tendon defects in rabbit Achilles (23) and patellar tendons (24, 25), blood derived stem cells (BDSCs) were used to successfully treat equine superficial digital flexor tendon injuries (26) and adipose derived stem cells (ASCs) to repair rabbit flexor tendons (27). Therefore, it is reasonable to presume that TSCs, which are tendon specific stem cells can be used to effectively repair tendon injuries. Besides, a comparison of properties between TSCs and MSCs, the most commonly used stem cells for biologics treatment, revealed that TSCs had higher clonogenicity, better proliferation potential, induced formation of more chondrocyte-like cells and higher accumulation of glycosaminoglycan than MSCs *in vitro*. TSCs also expressed higher levels of a stem cell marker (Oct-4), tenocyte markers (scleraxis, tenomodulin, decorin and collagen type I), chondrogenic markers (collagen type II and biglycan) and an osteogenic marker (alkaline phosphatase) (28).

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However, the beneficial effects of TSCs on tendon injuries have only started to emerge. Recently were TSCs shown to promote the repair of a patellar tendon window defect model in rats by increasing collagen production and improving the alignment of collagen fibers, Young's modulus and ultimate stress (29). In addition, transplantation of scleraxis-transduced TSCs promoted healing of a rat patellar tendon window injury at early stages (28). Furthermore, combining TSCs with PRP resulted in higher collagen type I mRNA synthesis in rats with Achilles tendon injury (30). Similar to TSCs, autologous tenocytes (likely a mixture of tenocytes and TSCs) in rabbits also augmented the healing and remodeling of rotator cuff tears (31), and

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improved histological outcomes and increased collagen content thereby healing chronic Achilles tendinopathy (32). More importantly, the tensile strength of the Achilles tendons was higher after treatment with both TSCs and PRP(32).

Treatment of tendon injuries using TSC therapy still is in a nascent stage. To become a successful therapy, a number of challenges have to be overcome. First, it is necessary to obtain a sufficient number of authentic TSCs for use in therapy. This challenge will be overcome by methods that prolong the stemness of TSCs in culture and expanding them effectively. A recent study reported that culturing TSCs along with insulin-like growth factor 1 (IGF-1) for 28 days retained multipotency in TSCs, and upregulated decorin and scleraxis expression (33). In addition, hypoxic culture conditions and low levels of PGE_2 could also enhance the stemness of TSCs in culture (34, 35). Second, the age of the donor might significantly affect the outcome of TSC treatments. Aging decreases the number of TSCs (21), and their proliferation potential and expression of stem cell markers (6) in animals. A 70% reduction in the number of TSCs was noted in ~ 24 months old rats when compared to ~ 3 months old rats (21), TSCs from 9 months old mice proliferated 3.5 times slower than TSCs from 2.5 months old mice and also had low levels of stem cell markers (6). Third, TSC therapy outcome may vary depending on TSC stemness, patient age, delivery techniques, etc. Moreover, the viability of the injected or implanted TSCs at present cannot be monitored. Therefore, new protocols and strategies to safely monitor the TSCs used for treatment should be developed. The popular mode of direct TSCs injection into the injured tendons for cell therapy may result in cell death and reduce the treatment efficacy. Therefore, it is desirable to use biocompatible carriers such as platelet-rich-

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plasma (PRP) along with TSCs to protect cells and stimulate them to proliferate and differentiate in the treatment sites.

Use of PRP to treat tendon injuries

PRP is now a popular method used to treat tendon injuries particularly in professional athletes. As its name indicates, PRP is rich in platelets containing numerous growth factors that are necessary for tissue healing. These include platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), fibroblastic growth factor (FGF), and hepatocyte growth factor (HGF) (36). Besides, PRP forms a tissue specific scaffold that is conducive for cell migration and new matrix formation (8, 37). As a promising healing agent, PRP is widely used orthopaedic surgery and sports medicine to augment the healing of injured musculoskeletal tissues, including tendons and ligaments (38).

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In clinical applications, PRP treatment via injections improved the pain intensity and functional ability scores in patients with elbow tendinopathy (46-48), Achilles tendinopathy (49, 50) (51)and patellar tendinopathy (52, 53).

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Besides PRP injections, PRP treatment can also be achieved by implantation of PRP gels, which may be superior to injections and yield better treatment outcomes because the PRP gel may stay in place without potential diffusion from the treated area. It has been reported that implanting PRP gel, called platelet-rich fibrin matrices (PRFM), into Achilles tendon tears in athletes improved the range of motion and function quicker than in those who received open suture repair (54). Similarly, implantation of PRFM along with acellular porcine dermal patch (APD) effectively healed acute Achilles tendon rupture in sheep by inducing the formation of new tendon fibers (55).

However, the efficacy of PRP on tendon injuries in clinical trials has not been consistent. A number of studies have also reported no benefits in the clinical outcomes after PRP treatment (49, 56, 57). These discrepancies are thought to be cause by two major factors; PRP-associated and patient-related (36). The PRP-associated factors include the following: 1) *PRP composition*: the presence or absence of white blood cells (WBCs) in PRP preparations; 2) *Platelet concentration*: low or high platelet concentration relative to the level in whole blood; 3) *PRP status:* activated or non-activated; 5) *Delivery method*: injection or implantation; and 6) *Number of PRP treatments*: one time injection or multiple injections. Patient-associated factors include: 1) *Age:* young or old; 2) *Type of tendon injury*: acute or chronic; 3) *Patient activity level*: active or passive; 4) *Treatment history*: prior treatments, surgeries, etc.; and 5) *Post-recovery plans*: rehabilitation or no-physiotherapy.

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Among the many factors influencing the clinical outcomes of PRP treatment for tendon injuries, the most important may be the age. This is because in aging patients, fewer stem cells are present in tissues. In tendons, the fewer TSCs may be of poor quality due to impaired proliferative ability and reduced stemness (6) that may diminish PRP treatment efficacy because PRP exerts its effects through its effect on TSCs; in other words PRP application alone is not sufficient to promote healing of injured tendons. Therefore, in aging patients, PRP treatment efficacy is not expected to be high. To mitigate this problem, it is recommended that aging patients perform moderate exercise to increase the number of stem cells. It has been shown that moderate exercise through treadmill running increases the number of TSCs in aging mouse tendons and improve the degenerative changes in aging tendons by decreasing lipid deposition, proteoglycan accumulation and calcification (6).

Another important factor affecting the efficacy of PRP treatment for tendinopathy (*i.e.* chronic tendon injury) is the disease stage of tendinopathy. Early stage tendinopathy is characterized by inflammation and/or nascent abnormal tissue differentiation (or formation of non-tendinous tissues at early stages) in the affected tendon. Therefore, PRP injection may be used to suppress tendon inflammation and hence reduce tendon pain, thus enhancing tendon function in patients. Indeed, the HGF in PRP was shown to have anti-inflammatory function (58) and PRP can suppress the non-tenocyte differentiation of TSCs at early stages (59).

However, when tendinopathy is in later stages, when the tendon is severely degenerated and contains lipid deposits, proteoglycan accumulation and calcification, either alone or in combination (60), PRP injection may be less effective because PRP itself cannot improve tendon

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degeneration. In this case, tissue debridement should be performed to improve the degenerative environment so that TSCs can self-renew and differentiate normally; that is, daughter TSCs and/or tenocytes can be produced because of corrected "niche" environment for TSCs (19, 61) (62). This, followed by PRP gel implantation is expected to improve tendon function in late stage tendinopathy (54).

It should be noted that studies that determine the efficacy of PRP treatment on tendon injuries (e.g. tendinopathy) in humans are also limited by unavoidable subjective evaluation of PRP treatment effects such as pain and functional scores by patients. Besides, as mentioned above, the patient population is typically heterogeneous due to differences in ages, gender, treatment history, activity level, etc. All these reduce the statistical power to detect the treatment effects when existent. Therefore, these limitations in human studies may be best addressed by performing well-controlled basic studies on animal models that produce more consistent results as discussed above.

Finally, a common theme stemming from previous PRP studies is that the use of ‘one’ PRP preparation in a commercial "one-size-fits-all" approach may not be optimal to treat all types of tendon injuries in patients of all ages. Patients can obtain the highest benefit from PRP treatments if the protocol is optimized based on individual age, treatment history, activity level and injury type.

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The combined use of TSCs and PRP to treat tendon injuries

Basic science studies on animal models show consistent outcomes of PRP treatment based on the cellular and molecular responses of tendons and tendon cells. Increase in the number of cells and collagen production were reported in tendons after PRP treatment (39-41). More relevant to this review is the impact of PRP on TSCs. PRP releasate was shown to induce anabolic differentiation of TSCs into tenocytes, which proliferated quickly and produced abundant collagen (39). In two recent studies, the effect of PRP along with TSCs was tested on injured or tendinopathic rat Achilles tendons. The results showed higher scleraxis, collagen type I and tenascin C mRNA in rats treated with a combination of PRP and TSCs indicating that combining the two components may have synergistic effects (30, 43). The tissue specific scaffold formed by PRP may stimulate TSC proliferation and differentiation into tenocytes thus augmenting the healing of injured tendons. This synergistic effect of PRP has also been reported for combination with cells and extracellular components. For example, injection of PRP with MSCs into rabbit mandibles improved bone formation, mineralization and the mechanical properties (44). Similarly, PRP, when combined with collagen, also significantly healed wounds in the porcine anterior cruciate ligament (ACL) and improved the load at yield, maximum load, and linear stiffness (45). These findings indicate that the tissue scaffold formed by PRP could enhance the healing ability of stem cells in the treatment of tissue injuries.

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Concluding Remarks

Tendon injuries are common in both athletic and occupational settings. Current treatments are however ineffective and cannot restore the normal tendon structure and function effectively. Therefore, alternative methods are eagerly pursued. Biologics treatments such as cell therapy with TSCs and cell free therapy with PRP have the potential to effectively repair or even regenerate tendons after injury. Challenges facing TSC therapy for tendon injuries are numerous, including the generation of sufficient number of authentic TSCs *in vitro* and the optimal means to deliver TSCs to the injury site so that cells are survival and functional in the new healing environment. On the other hand, autologous PRP is already in use in clinics for the treatment of tendon injuries. PRP provides a natural conductive scaffold, and also contains abundant growth factors (e.g. PDGF, TGF-β, VEGF, IGF, and HGF), which can enhance healing of injured tendons. PRP treatment was shown to induce TSC differentiation into active tenocytes, which proliferate quickly and produce abundant collagen, indicating the potential of PRP to enhance the repair of injured tendons (39). Therefore, the combined use of TSCs and PRP has great potential for effective cell therapy of tendon injuries.

Moreover, PRP was also shown to exert anti-inflammatory effects on injured tendons (58), which may explain why PRP injections can reduce tendon pain. However, the efficacy of PRP treatment for tendon injuries remains a hotly debated topic in orthopaedic surgery and sports medicine. The efficacy issue of PRP is believed to be due to PRP-associated factors and patient-related factors (36). Therefore, a PRP application approach tailored for individual needs instead of the current "one-size-fits-all" approach should be used in clinics to treat tendon injuries. Lastly, the rehabilitation protocol following such biologics treatments should also be

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customized to an individual to promote full recovery of tendons; in fact, it is a prerequisite to apply mechanical loading on healing tendons in order for PRP to enhance the healing outcome of injured tendons (63).

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REFERENCES

1. Kujala UM, Sarna S, Kaprio J: Cumulative incidence of achilles tendon rupture and tendinopathy in male former elite athletes. Clin J Sport Med 15: 133-135, 2005
2. Scott A, Ashe MC: Common tendinopathies in the upper and lower extremities. Curr Sports Med Rep 5: 233-241, 2006
3. Lian OB, Engebretsen L, Bahr R: Prevalence of jumper's knee among elite athletes from different sports: a cross-sectional study. Am J Sports Med 33: 561-567, 2005
4. de Jong JP, Nguyen JT, Sonnema AJM, et al: The Incidence of Acute Traumatic Tendon Injuries in the Hand and Wrist: A 10-Year Population-based Study. Clinics in Orthopedic Surgery 6: 196-202, 2014
5. Milgrom C, Schaffler M, Gilbert S, et al: Rotator-cuff changes in asymptomatic adults. The effect of age, hand dominance and gender. J Bone Joint Surg Br 77: 296-298, 1995
6. Zhang J, JH W: Moderate exercise mitigates the detrimental effects of aging on tendon stem cells. PLoS ONE 2015

Accepted manuscript

ACCEPTED MANUSCRIPT

7. Morais DS, Torres J, Guedes RM, et al: Current Approaches and Future Trends to Promote Tendon Repair. Ann Biomed Eng 43: 2025-2035, 2015

8. Wang JH: Mechanobiology of tendon. J Biomech 39: 1563-1582, 2006

9. Zhang J, Wang JH: The Effects of Mechanical Loading on Tendons - An In Vivo and In Vitro Model Study. PLoS ONE 8: e71740, 2013

10. Buchanan CI, Marsh RL: Effects of long-term exercise on the biomechanical properties of the Achilles tendon of guinea fowl. J Appl Physiol 90: 164-171, 2001

11. Andres BM, Murrell GA: Treatment of tendinopathy: what works, what does not, and what is on the horizon. Clin Orthop Relat Res 466: 1539-1554, 2008

12. Hadi M, Young J, Cooper L, et al: Surgical management of chronic ruptures of the Achilles tendon remains unclear: a systematic review of the management options. Br Med Bull 108: 95-114, 2013

13. Shelbourne KD, Gray T, Haro M: Incidence of subsequent injury to either knee within 5 years after anterior cruciate ligament reconstruction with patellar tendon autograft. Am J Sports Med 37: 246-251, 2009

14. Decloedt E, Blockman M, Vol. 28 237-2382010).

15. Ziltener JL, Leal S, Fournier PE: Non-steroidal anti-inflammatory drugs for athletes: An update. Annals of Physical and Rehabilitation Medicine 53: 278-288, 2010

16. Selfe J, Alexander J, Costello JT, et al: The effect of three different (-135 degrees C) whole body cryotherapy exposure durations on elite rugby league players. PLoS ONE 9 2014

Accepted manuscript

ACCEPTED MANUSCRIPT

17. Hausswirth C, Louis J, Bieuzen F, et al: Effects of whole-body cryotherapy vs. far-infrared vs. passive modalities on recovery from exercise-induced muscle damage in highly-trained runners. PLoS ONE 6: 7, 2011

18. Mayer F, Hirschmuller A, Muller S, et al: Effects of short-term treatment strategies over 4 weeks in Achilles tendinopathy. Br J Sports Med 41: 29, 2007

19. Bi Y, Ehirchiou D, Kilts TM, et al: Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med 13: 1219-1227, 2007

20. Zhang J, Wang JH: Characterization of differential properties of rabbit tendon stem cells and tenocytes. BMC Musculoskelet Disord 11: 1471-2474, 2010

21. Zhou Z, Akinbiyi T, Xu L, et al: Tendon-derived stem/progenitor cell aging: defective self-renewal and altered fate. Aging cell 9: 911-915, 2010

22. Rui YF, Lui PP, Li G, et al: Isolation and characterization of multipotent rat tendon-derived stem cells. Tissue Eng Part A 16: 1549-1558, 2010

23. Young RG, Butler DL, Weber W, et al: Use of mesenchymal stem cells in a collagen matrix for achilles tendon repair. Journal of Orthopaedic Research 16: 406-413, 1998

24. Hani AA, David LB, Gregory PB, et al: Autologous Mesenchymal Stem Cell-Mediated Repair of Tendon. Tissue Engineering 5: 267-277, 1999

25. Juncosa-Melvin N, Boivin GP, Gooch C, et al: The effect of autologous mesenchymal stem cells on the biomechanics and histology of gel-collagen sponge constructs used for rabbit patellar tendon repair. Tissue Eng 12: 369-379, 2006

26. Marfe G, Rotta G, De Martino L, et al: A new clinical approach: Use of blood-derived stem cells (BDSCs) for superficial digital flexor tendon injuries in horses. Life Sciences 90: 825-830, 2012

Accepted manuscript

ACCEPTED MANUSCRIPT

27. Kryger GS, Chong AK, Costa M, et al: A comparison of tenocytes and mesenchymal stem cells for use in flexor tendon tissue engineering. J Hand Surg Am 32: 597-605, 2007

28. Tan J, Wu W, Xu X, et al: Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: A randomized controlled trial. JAMA 307: 1169-1177, 2012

29. Ni M, Lui PPY, Rui YF, et al: Tendon-derived stem cells (TDSCs) promote tendon repair in a rat patellar tendon window defect model. Journal of Orthopaedic Research 30: 613-619, 2012

30. Chen L, Dong S-W, Liu J-P, et al: Synergy of tendon stem cells and platelet-rich plasma in tendon healing. Journal of Orthopaedic Research 30: 991-997, 2012

31. Chen JM, Willers C, Xu J, et al: Autologous tenocyte therapy using porcine-derived bioscaffolds for massive rotator cuff defect in rabbits. Tissue Eng 13: 1479-1491, 2007

32. Chen J, Yu Q, Wu B, et al: Autologous tenocyte therapy for experimental Achilles tendinopathy in a rabbit model. Tissue Eng Part A 17: 2037-2048, 2011

33. Holladay C, Abbah S-A, O'Dowd C, et al: Preferential tendon stem cell response to growth factor supplementation. Journal of Tissue Engineering and Regenerative Medicine: n/a-n/a, 2014

34. Zhang J, Wang JH: Human tendon stem cells better maintain their stemness in hypoxic culture conditions. PLoS ONE 8 2013

35. Zhang J, Wang JH: Prostaglandin E2 (PGE2) exerts biphasic effects on human tendon stem cells. PLoS One 9: e87706, 2014

36. Wang JHC, Zhang J, Nirmala X: Advancements in the Treatment and Repair of Tendon Injuries. Current Tissue Engineering 3: 71-81, 2014

Accepted manuscript

ACCEPTED MANUSCRIPT

37. Molloy T, Wang Y, Murrell G: The roles of growth factors in tendon and ligament healing. Sports medicine 33: 381-394, 2003

38. Foster TE, Puskas BL, Mandelbaum BR, et al: Platelet-rich plasma: from basic science to clinical applications. The American journal of sports medicine 37: 2259-2272, 2009

39. Zhang J, Wang JH: Platelet-rich plasma releasate promotes differentiation of tendon stem cells into active tenocytes. Am. J. Sports Med. 38: 2477-2486, 2010

40. Anitua E, Andia I, Sanchez M, et al: Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. J Orthop Res 23: 281-286, 2005

41. Anitua E, Sanchez M, Nurden AT, et al: New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol 24: 227-234, 2006

42. Kaux JF, Drion PV, Colige A, et al: Effects of platelet-rich plasma (PRP) on the healing of Achilles tendons of rats. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 20: 748-756, 2012

43. Chen L, Liu JP, Tang KL, et al: Tendon derived stem cells promote platelet-rich plasma healing in collagenase-induced rat achilles tendinopathy. Cell Physiol Biochem 34: 2153-2168, 2014

44. Hwang YJ, Choi JY: Addition of mesenchymal stem cells to the scaffold of platelet-rich plasma is beneficial for the reduction of the consolidation period in mandibular distraction osteogenesis. J Oral Maxillofac Surg 68: 1112-1124, 2010

45. Murray MM, Spindler KP, Abreu E, et al: Collagen-platelet rich plasma hydrogel enhances primary repair of the porcine anterior cruciate ligament. J Orthop Res 25: 81-91, 2007

Accepted manuscript

ACCEPTED MANUSCRIPT

46. Mishra A, Pavelko T: Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med 34: 1774-1778, 2006

47. Peerbooms JC, Sluimer J, Bruijn DJ, et al: Positive effect of an autologous platelet concentrate in lateral epicondylitis in a double-blind randomized controlled trial: platelet-rich plasma versus corticosteroid injection with a 1-year follow-up. The American journal of sports medicine 38: 255-262, 2010

48. Thanasas C, Papadimitriou G, Charalambidis C, et al: Platelet-rich plasma versus autologous whole blood for the treatment of chronic lateral elbow epicondylitis: a randomized controlled clinical trial. Am J Sports Med 39: 2130-2134, 2011

49. de Vos RJ, Weir A, van Schie HT, et al: Platelet-rich plasma injection for chronic Achilles tendinopathy: a randomized controlled trial. JAMA 303: 144-149, 2010

50. Hechtman KS, Uribe JW, Botto-vanDemden A, et al: Platelet-rich plasma injection reduces pain in patients with recalcitrant epicondylitis. Orthopedics 34: 92, 2011

51. Filardo G, Kon E, Di Matteo B, et al: Platelet-rich plasma injections for the treatment of refractory Achilles tendinopathy: results at 4 years. Blood Transfus 12: 533-540, 2014

52. Filardo G, Kon E, Della Villa S, et al: Use of platelet-rich plasma for the treatment of refractory jumper's knee. Int Orthop 34: 909-915, 2010

53. Jeong DU, Lee C-R, Lee JH, et al: Clinical Applications of Platelet-Rich Plasma in Patellar Tendinopathy. BioMed Research International 2014: 15, 2014

54. Sánchez M, Anitua E, Azofra J, et al: Comparison of Surgically Repaired Achilles Tendon Tears Using Platelet-Rich Fibrin Matrices. The American Journal of Sports Medicine 35: 245-251, 2007

Accepted manuscript

ACCEPTED MANUSCRIPT

55. Sarrafian TL, Wang H, Hackett ES, et al: Comparison of Achilles tendon repair techniques in a sheep model using a cross-linked acellular porcine dermal patch and platelet-rich plasma fibrin matrix for augmentation. J Foot Ankle Surg 49: 128-134, 2010

56. de Jonge S, de Vos RJ, Weir A, et al: One-year follow-up of platelet-rich plasma treatment in chronic Achilles tendinopathy: a double-blind randomized placebo-controlled trial. Am J Sports Med 39: 1623-1629, 2011

57. Schepull T, Kvist J, Norrman H, et al: Autologous platelets have no effect on the healing of human achilles tendon ruptures: a randomized single-blind study. Am J Sports Med 39: 38-47, 2011

58. Zhang J, Middleton KK, Fu FH, et al: HGF Mediates the Anti-inflammatory Effects of PRP on Injured Tendons. PlosOne 8: e67303, 2013

59. Zhang J, Wang JH: PRP treatment effects on degenerative tendinopathy - an in vitro model study. Muscles Ligaments Tendons J 2014 4: 10-17. eCollection 2014 Jan.,

60. Kannus P, Jozsa L: Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. J Bone Joint Surg Am 73: 1507-1525, 1991

61. 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 32: 6972-6981, 2011

62. Becerra J, Santos-Ruiz L, Andrades J, et al: The Stem Cell Niche Should be a Key Issue for Cell Therapy in Regenerative Medicine. Stem Cell Rev and Rep 7: 248-255, 2011

63. Virchenko O, Aspenberg P: How can one platelet injection after tendon injury lead to a stronger tendon after 4 weeks? Interplay between early regeneration and mechanical stimulation. Acta orthopaedica 77: 806-812, 2006

Accepted manuscript