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Design and Analysis on platelet-rich plasma for Tendon healing in Rats

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ABSTRACT

To treat tendon injuries, platelet-rich plasma (PRP) is widely utilized in orthopedic surgery and sports medicine. Tendinopathy, a condition of the tendons, can be treated using PRP. The purpose of this study is to examine the applicability of the combination of three different cell types and PRP on tenocyte-conditioned medium (CM) to investigate the potential effect on promoting tendon healing. Tenocytes, bone marrow mesenchymal stem cells and dermal fibroblasts were cultured in a three-dimensional PRP hydrogel with or without CM, and the proliferation and migration of the cells were observed. Through reverse transcription quantitative polymerase chain reaction and enzyme-linked immunosorbent assay, the CM derived from tendon was applied to mesenchymal stem cells, tendons, and skin fibroblasts, respectively, to determine its effect on matrix formation phenotype and inflammatory protein secretion. Tendon cell CM combined with PRP stimulated tendon formation of mesenchymal stem cells and skin fibroblasts and reduced the secretion of inflammatory proteins. In conclusion modifying the target tissue with PRP before cell implantation can optimize the effect of cell therapy. Skin fibroblasts and bone marrow mesenchymal stem cells combined with PRP can be used to regenerate tendons.

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Introduction:

Using stem cells in conjunction with platelet- rich plasma therapy

Stem cells conjunction with Platelet Rich Plasma Therapy have been studied extensively in both animal and human research as a way to enhance tissue regeneration in severe degeneration. An article in the Journal of Translational Medicine published in 2017 demonstrated that intra- articular injections of fresh stromal vascular fraction cells (a source of fat-derived stem cells) paired with PRP exhibited healing potential in rats with degenerative osteoarthritis.

"This is in line with previously published results from both preclinical and clinical investigations," the researchers wrote in their paper. As a result, rats' degenerative osteoarthritis significantly improved, resulting to a higher quality of life for them. Clinically considerable pain relief was one of these improvements. Therefore, SVF (adipose stem cells) paired with platelet-rich plasma (PRP) may be can an effective treatment for orthopedic disorders when combined with PRP. Using PRP as a growing medium for stem cells has been studied for years. PRP is a bioactive scaffold that can release endogenous growth factors, according to a 2012 study by doctors at Shanghai Jiaotong University School of Medicine in China. Bone-marrow stem cells and adipose- derived stem cells were able to transform into chondrocytes (cartilage building blocks) when seeded within the PRP scaffold. Dense tissue called tendons joins muscles to bones. The muscles convey muscle forces to the bones, allowing the joints to move as a result. A large amount of mechanical stress is placed on tendons, which can cause injury and affect their function. Every year, roughly 16.4 million

Americans seek medical treatment for tendon and ligament injuries in orthopaedic clinics (1, 2). Toxic overuse is most likely to cause injury to the Achilles, patella, rotator-cuff, and forearm extensor tendons (3). As a rule, tendons have low blood flow. It has been shown that hypervascularity and disorder in vessel distribution are present in chronic tendon damage or tendinopathy, which may alter the mechanics, and cause pain. Agrowing number of musculoskeletal disorders, including tendinopathy, are being treated with Platelet- Rich Plasma (PRP) in recent years. US and European physicians currently use PRP for acute or chronic tendon injuries as well as ligament or muscle tears. PRP's market worth is expected to reach $126 million this year due to its widespread use (4). Anucleate cytoplasmic pieces called platelets are the main components of PRP, which are generated in the bone marrow by megakaryocytes.

1. LITERATUREREVIEW

Approximately 40–50 percent of athletes suffer from tendon injuries (1), which are common in athletic situations. Injury to the tendons, however, is also widespread in the workplace and in the elderly population. (5). It is estimated that 15 percent of people aged 50-59 and 51 percent of those aged 80 and above suffer from tendon injuries (6). Sixty to eighty percent of tendon cells are made up of collagen type I and elastin (7,8). Strain-resisting tendons connect bones to muscles for the same reason. Acute tendon injuries are caused by severe or repetitive stresses (2, 9–7).

Cellular activity in tendons are altered when subjected to aberrant loading circumstances, leading to structural changes that ultimately limit tendon function. Acute and chronic tendon injuries are the most common types of tendon injuries. Mechanics overloading the tendon leads to acute tendons injury. It is believed that

chronic injuries, which are also known as tendinopathies, are mostly caused by mechanical overuse of the tendon. Naturally, the injured tendon heals. It's a lengthy and inefficient process that does not restore the wounded tendon's typical biological and biomechanical characteristics. Therefore, rats are more commonly unable to resume their pre- injury activities (10, 11). The mended area is also more susceptible to re-injury, especially in returning athletes (4).

No consensus exists on how to treat and manage tendon injuries despite their prevalence. Some of the most common therapies are traditional, such as nonsteroidal anti-inflammatory medications (NSAIDs), cryotherapy (12, 13), physiotherapy and so on. To restore normal tendon structure and function after an injury, improved therapeutic alternatives are urgently needed.

PRP is already a useful tonic for promoting tendon-derived stem cells in active tendon cells in the laboratory, and that TDSCs implant treatments, alone or with PRP, accelerate the healing of ruptured achilles tendon in the early stages (14)

2. USE OF (PRP) TO TREAT TENDON IN JURIES

Tendon injuries, especially in professional sports, are being treated using PRP. PRP, as its name implies, contains a number of growth factors important for tissue healing. Many other growth factors exist, such as the platelet-derived growth factor, the transformative growth factor, the vasculature-endothelial-growth factor, the epidermal growth factor (EGF), the Insulin-Like growth factor 1, the FGF, as well as the liver growth factor (HGF). Furthermore, PRP creates an environment favourable to cell migration and the production of new collagen. Sports medicine and orthopaedic surgery use PRP as an effective healing agent for tendons and

ligaments that have been injured. Elbow tendons, Achilles tendons, and patellar tendons were treated with PRP injections, which reduced the severity of symptoms and improved functional abilities.

For rats who do not want to undergo injections or are unable to tolerate the pain associated with injections, PRP gel implants may be a better option. An athlete's Achilles tendon rupture can be treated more quickly with platelet-rich fibrin matrices (PRFMs) than with open suture healing. By stimulating the growth of new tendon fibers following their implantation, PRFM and APD have been proven to effectively treat acute Achilles tendon ruptures in sheep as well.

When it comes to tendon injuries, PRP's usefulness in clinical trials isn't always crystal cut. There have been a lot of studies that have found no advantage from PRP treatment in terms of clinical outcomes as well. Both PRP- related and rats-related factors are likely to be responsible for these disparities. PRP is connected with the following factors: This includes: WBC content, PRP activation state, injection or implantation, number of PRP treatments (one injection or repeated injections), platelet concentration (low or high) relative to total blood, PRP delivery approach (injection or implantation). Age, kind of injury, rats activity level, treatment history, and post- recovery plans are just a few of the rats-related aspects. PRP treatment for tendon injuries is affected by a variety of factors, but one of the most crucial is the rat's age. A rat's tissues have a smaller number stem cells as they age. Since TSCs account for most of PRP's effects, tendons with fewer TSCs may be of poorer quality due to their limited proliferative potential and stemness. As a result, the efficacy of PRP treatment in older adults is not expected to be substantial. A reasonable quantity of exercise is recommended for those over the age of 65 in order to

enhance the number of stem cells. In aging mouse tendons, moderate treadmill jogging increases the number of TSCs. It also reduces lipid deposits, proteoglycan buildup, and calcium deposits.

3. METHODS Cell Expansion

Anterior cruciate ligament reconstruction was performed on three volunteers, all of whom had semitendinosus tendon biopsies. Explant culture was used to isolate tenocytes. As well as 4 male and female SD rats (200g). Cells were grown in DMEM with 10 percent FBS and skin fibroblasts at 37°C in 5 percent CO2 (Hyclone; GE Healthcare). Using trypan blue, the viability of the cells was determined before plating.

Preparation of PRP

After providing informed agreement, participants in the control group of a randomized PRP clinical trial had their peripheral blood collected into citrate tubes (Vacuette; Greiner BioOne). PRP was made by centrifuging at 570 g for 7 minutes in a single-step centrifuge. In comparison to peripheral blood, this approach increased platelet concentration by 1.84 0.42– fold, and more than 99 percent of red blood cells and white blood cells were removed from the sample as a result of the process.

Preparation of Tenocyte-CM

PRP hydrogel cultures in T75 flasks were used to prepare the tenocyte-CM. 10 percent PRP was added to the culture mix, and 3D PRP hydrogels were used to sustain the cells at 37°C and 5 percent CO2 for 96 hours. When the cells were spun down, they yielded concentrated medium, or CM, which was used as a supplement in 3D cultures of rat BM-MSC cells as well skin- fibroblasts and Tenocytes cells. Their indirect

cocultures of BMMSC, skin, and tendon fibroblasts showed a positive response to this particular tendon cell culture (indicating preconditioning the host tissue with PRP).

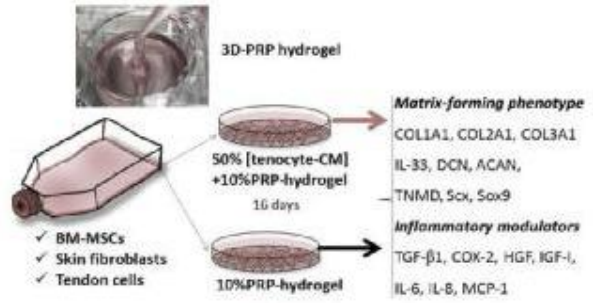


Figure 1. indirect coculture with tenocyte-CM

Hydrogel Cultures and Treatments

After fasting for 24 hours before the experiment, trypsinization was utilized to extract the larger cells (TryPLE select; Gibco, Life Technologies). It was decided to either obtain samples of each cell's phenotype for constitutive gene expression, or to use 3Dplasma hydrogels with 4 104 cells per well as reported before. 22 Because of Ca2+ in the culture media (1.05mM), the synthesis of thrombin occurs, followed by fibrinogen dissociation and the generation of fibrin dimers. When fibrin forms, cells are encapsulated in the gel. Accordingly, DMEM was added to each well at a ratio of 10:50:1, along with PRP and CM. In addition, PRP hydrogels maintain liquid CM in place when the clots. A 10 percent PRP-DMEM solution was used to test the effects of the CM on additional wells in the study. During the 15-day period, cells were cultivated in 3Dhydrogels. The extruded supernatant was changed every 3 days with media and PRP or media and PRP with CM,

depending on the condition. There were three PRP donors, three skin donors (three) and three tendon donor (three) as well as tenocytes from three separate donors employed in the parallel experimentation (Figure 1).

TABLE 1
Abbreviated Terms

ACAN	Aggrecan
BM-MSCs	Bone marrow–derived mesenchymal stem cells
CM	Conditioned media
COL1A1	Collagen type I alpha 1
COL2A1	Collagen type II alpha 1
COL3A1	Collagen type III alpha 1
COX-2	Cyclooxygenase 2
DCN	Decorin
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HGF	Hepatocyte growth factor
IGF-1	Insulin-like growth factor–1
IL33	Interleukin-33
IL-6	Interleukin-6 (protein)
PRP	Platelet-rich plasma
Scx	Scleraxis
Sox9	SRY Box 9
TBP	TATA box binding protein
TGF-b1	Transforming growth factor–beta 1
TNMD	Tenomoduli

TABLE 2
Doubling Times of Tenocytes, BM-MSCs, and Skin Fibroblasts Under Different Experimental Conditions[a]
Doubling Time, h

	PRP	CM	DMEM
Tenocytes	34.35 (4.50)	50.25 (2.08)	No proliferation
BM-MSCs	37.34 (3.37)	40.36 (3.37)	73.01 (10.54)
Skin fibroblasts	36.78 (3.37)	41.09 (8.92)	82.39 (49.60)

[a]Results are shown as the mean (SEM) of the experimental duplicates. DMEM, Dubelcco modified Eagle medium.

4. RESULTS

CM Induces Cell Proliferation

It is seen in Figure 2 that the 3 cell phenotypic populations moved toward CM gradients (Rayleigh test significance). In the CM gradient, BM-Mesenchymal stem cells had higher directness (directionality), which is defined as displacement divided by the entire route length of the cell (P 14.202). The COM migrated at the same speed and distance regardless of cell type.

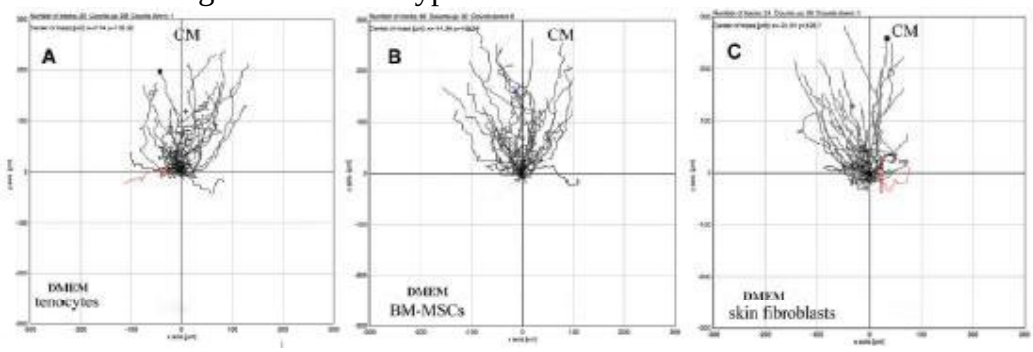
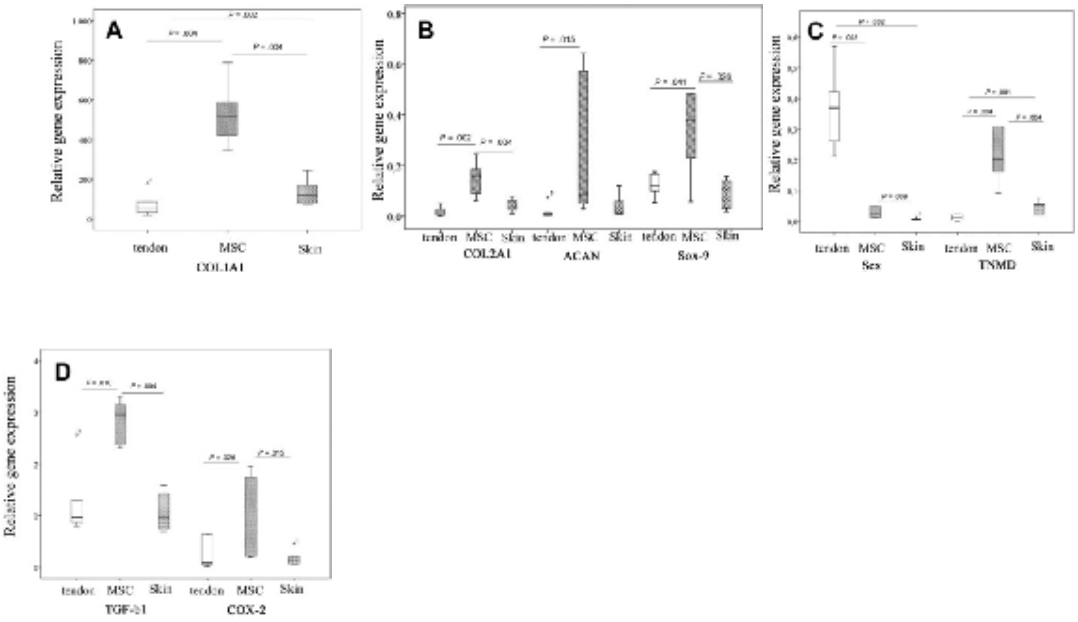


Figure 2. Tenocyte (A), BM-MSC (B), and skin fibroblast migration (C) over 24 hours toward a gradient produced by CM (n 30). Diffusion creates the gradient by injecting CM at the upper portion of the m- slide and allowing it to slowly diffuse down the slide. DME, Dubelcco modified Eagle medium, is a medium that contains Dubelcco modified Eagle bacteria.



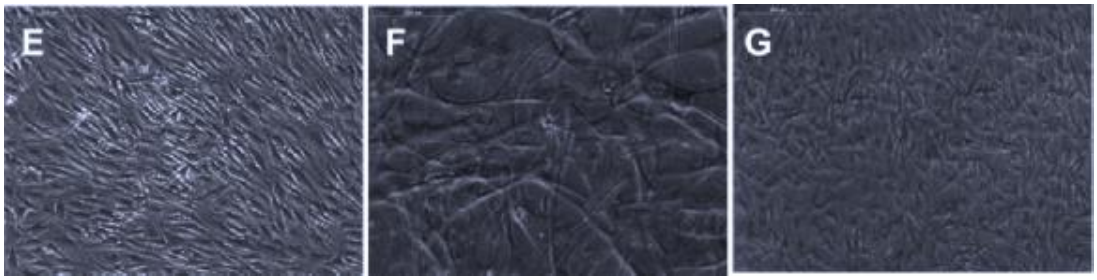


Figure 3. Analyzing gene expression by reverse transcription quantitative polymerase chain reaction in 3D platelet rich plasma hydrogels (A, B, and C), as well as inflammasome activator genes (D). Tenocytes,
BM-MSC and skin-fibroblasts were cultured for 15 days in the 3D hydrogels. at the 2 DCt -relative gene expression box plots, you'll see median values and 25th to 75th percentiles. 3D PRP hydrogel phase contrast photos of (E) tenocytes, (F) BM-MSC-derived cells, and (G) skin fibroblasts are shown. Lengthening.

There is a difference in gene expression. Tenocytes, BM-Mesenchymal stem cells, and skin fibroblasts were cultured in 3D PRP hydrogels for 15 days and their matrix-forming phenotypes were different (Figure 3). There was a 4-fold difference in the relative expression of COL1A1 between BM-Mesenchymal stem cells and skin fibroblasts (P14.014), and a 7-fold difference between tenocytes (P 14.014). A higher amount of COL1A1 was found in skin fibroblasts than in tenocytes (P =.002) (Figure 3A). On the other hand, BM-Mesenchymal stem cells exhibited larger quantities of COL2A1, ACAN, and Sox9 than tenocytes or skin fibroblasts in PRP hydrogels, and there were no differences between skin and tenocytes in terms of cartilage molecules (Figure 3B). (P .004) The expression of TNMD in BM-Mesenchymal stem cells was higher than in other cell types (Figure 3C). They both showed favorable associations with TNM (r 14 0.727, P 14.001) and with TNM- D(r 14 0.95, P 14.011), according to the data. For both anatomic cell sources, the levels of Scx expression were substantially higher in tenocytes (P 14.012). (Figure 3C). (P 14.0026 and P 14.0015, respectively) (Figure 3D).

Humoral Growth Factor (HGF) was expressed at moderately low levels in skin, tenocytes, and BM-Mesenchymal stem cells. As a result, we found that tenocytes expressed higher quantities of IGF-1 (P 14.026), and there was a positive correlation between IGF-1 and Scx expression levels (r 14 0.565, P 14.014). changes in matrix formation phenotype and growth factor expression induced by CM are discussed.

Aspindle-shaped tenocyte morphology was seen in Figures 3 and 4 (Figure 3E) in the absence of collagen matrix (CM) (Figure 4C). According to Figure 4, F and I (left), the spindle shape was observed in both Mesenchymal stem cells and skin cells after 15 days of exposure to CM, but not in those exposed to PRP hydrogels without CM (Figure 3, F and G). BM-MSCs showed higher expression of collagen I, the major fibrous component of the extracellular tendon matrix, and had the largest COL I: COL III ratio.

5. DISCUSSION

Based on biological interventions implanted in vitro amplified cells such as skin fibroblasts, tendon cells, or BM-Mesenchymal stem cells, binding PRP is a potential strategy for tendon repair. (15,16) In order to improve the results, it is necessary to study the optimal cell source, delivery and regulation of pre-implantation host tissue. Pretreatment of target tendons with PRP injection can stimulate cell proliferation, conversation and secretion activities of endogenous tendon cells (i.e. CM). As shown in this study, PRP combined CM enhances tendon production of skin fibroblasts and bone marrow- filled stem cells. Therefore, after pretreatment of the target tendon with PRP injection, implanting skin fibroblasts or BM-Mesenchymal stem cells in combination with PRP will have more opportunities to regenerate the tendon.

Bone marrow interstitial stem cells have advantages because they are more synthetically active and less inflamed. Depending on the anatomical source, cells can react differently to different molecular environments by altering the matrix to form phenotypes and the

secretion of inflammatory proteins. In vitro plasticity provides insight into the behavior of these cell groups when they transport PRP to the tendon. A common problem with local implantation of cells is that most cells do not remain in the target tissue and are lost. (15) To solve this critical problem, cells can be transported with PRP and confined to selected locations. In fact, cells injected into liquid vectors can spread to surrounding tissues. Conversely, when activated, PRP is pasted, including cells in the PRP stent. Local cell migration and proliferation are important issues to consider when designing biological therapy. PRP has been shown to have the characteristics of tendon cells with tendons that are more convergent and fissiony.

The CM of PRP-processed tendon cells is also tendonized and silky, and can induce endogenous cell migration. However, additional exogenous cells may need to be implanted in the treatment of serious diseases. The optimal cell source of tendon conditions may depend on whether the lesion is at the midpoint of the tendon or at the point of attachment of fibrous cartilage. In this regard, bone marrow interstitial stem cells express higher levels of cartilage matrix formation molecules (collagen 2, ACAN and transcription factor Sox-9) and TNDM than tendon cells or skin fibroblasts. TNDM is an angiogenesis inhibitor that is mainly expressed in the vascular-free areas of tendons and cartilage. Although the problem cannot be based on gene expression data and requires mandatory in vivo confirmation, in vitro results may indicate a combination of BMMesenchymal stem cells PRP may be a suitable method for the attachment end of recycled fiber cartilage, through which fiber cartilage adheres to the bone. In the body, a group of rats injected with BM-Mesenchymal stem cells showed better end tissue and biomemeological properties than the control group or rats with impaired ends after surgery to cut off the end of the Achilles tendon Treated with cartilage cells. Although this surgical cross-sectional model does not reflect overuse

or inherent tendon degeneration, it illustrates the healing potential of BM-MSC in tendons. Skin fibroblasts and bone marrow-filled stem cells in PRP hydrogels express different levels of Scx than tendon cells, so they are not considered to differentiate into tendon cells. Scx is a key transcription factor expressed in tendon ancestral cells and tendon cells.

The molecular microenframe provided by CM (rather than the PRP environment) stimulates the expression of Scx in BM-Mesenchymal stem cells and skin fibroblasts, consistent with other coccitrating models involving Mesenchymal stem cells and tendon cells. Then challenges in the field of cell-based regenerative therapies, discussing the role of cancer stem cells in promoting tendon regeneration, particularly through their ability to enhance the tendon characteristics of cells residing in tendons. (16,17) Bone marrow interstitial recharged stem cells showed higher expression of type I collagen, which is the main fiber component of the extracellular matrix of tendons, with the largest ratio of type I collagen to type III collagen. Tendons are mechanically functional tissues, and the low proportion of type I collagen and type III collagen is associated with weaker mechanical properties. (18) supports these data, BM-MSC implanted with tendon lesions naturally occurring in the shallow flexor tendon of the finger, and during the six-week follow-up, BM-MSC enhanced the normalization of biochemical and component parameters without fibrosis. One disadvantage of bone marrow interstellation stem cells is that when they are exposed to CM, the ratio of type I collagen to type III collagen decreases. In this case, skin fibroblasts can be more strongly involved in the activation of tendon healing in the matrix, as in addition to tendon markers, they also show a convenient ratio of type I collagen to type III collagen.

As a result, skin fibroblasts may be more appropriate than bone marrow-filled stem cells, provided that the tendon is regulated with PRP before the

cell is implanted. Further confirmation of these results is necessary in animal models with naturally occurring tendon lesions. Typically, research does not focus on changing target tissue to optimize the effectiveness of cell therapy. One possibility is that the host tissue does not receive transplanted cells and does not provide normal nutritional signals due to inflammation. Another weakness of the study is that no mechanism has been explored. Despite observations suggesting that the three cell types behave differently, we still don't know why they behave differently. To improve our basic scientific understanding of process signaling pathways such as IL-1R and kB nuclear agent, activation must be explored.

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In order to draw reliable conclusions, these 3 cell types must be tested in another animal models of tendon lesions, and the resulting regenerative tendon composition and biomemetric properties can confirm the selection of the most effective cell type for appropriate tendon or end regeneration. Finally, only a single formulation of PRP was tested, and there were other clinical options.

CONCLUSION

We've discussed how the type of cells used in tendon restoration can affect the biological parameters involved. When BM-Mesenchymal stem cells and skin fibroblasts were coupled with PRP, tenogenesis was stimulated. It has been found that BM-Mesenchymal have a higher collagenI: collagen III ratio, they appear to be a better alternative for treating tendon lesions than BM-Mesenchymal stem cells. These findings have implications for the design of clinical studies involving

autologous cells and PRP (platelet rich plasma).

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