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. This study offers a review of basic scientific investigations on PRP that have been undertaken under controlled conditions. Research shows that PRP has anabolic and anti-inflammatory benefits on tendons, and this has been proven in numerous studies to be true. After the rupture of the Achilles tendon, a mouse study will test the idea that platelet rich plasma can activate tendon-derived stem cells (TDSCs). The in vitro study showed that PRGF (activated PRP) greatly increased cell DNA synthesis, viability, and proliferation, while also boosting cell migration and recruitment of TDSCs, as an example. It is important to note that there are also CTCs (collagen PRP TDSC constructions) as well as PRP/collagen TDSC constructs (PCTCs). As well as CTCs, PCTCs also retained their viability after three weeks of in vitro culture, with the PCTCs displaying a better alignment than the CTCs. Its microstructure also revealed more visible fiber-like tissues and generated a microvascular system that was more cyclic.

1. INTRODUCTION

Combining stems cells with Platelet Rich Plasma Therapy

Stem cells combined 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 patients 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, patients' degenerative osteoarthritis significantly improved,
resulting to a higher quality of life for them. Clinically considerable pain relief was one of these improvements.

The study revealed:

- This improvement was maintained at the two-year mark in all individuals with osteoarthritic knees.
- Three to six months after receiving SVF and PRP injections, a small percentage of patients demonstrated improvement.
- So, regenerative medicine's mode of action is a chain reaction that takes place over time.
- As a result of these processes, the immune system can be modulated, leading to tissue remodeling.
- It's possible that the stem cells' soluble growth factors are responsible for anti-inflammatory and pain-relieving benefits.
- In 8 out of 10 patients, the stem cell and PRP injections greatly reduced the need for pain medication.
- A two-year follow-up showed no major negative effects for the researchers.

Finally, SVF (adipose stem cells) paired with platelet-rich plasma (PRP) may be an effective treatment for orthopedic disorders when combined with PRP. 1

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.

A growing 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 [13]. Anucleate cytoplasmic pieces called platelets are the main components of PRP, which are generated in the bone marrow by megakaryocytes.

2. LITERATURE REVIEW

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. According to hospital records from 2001 to 2010, hand and wrist tendon injuries accounted for nearly 25
percent of all work-related injuries in Olmsted County in Minnesota. (4). It is estimated that 15 percent of people aged 50-59 and 51 percent of those aged 80 and above suffer from tendon injuries (5).

Sixty to eighty percent of tendon cells are made up of collagen type I and elastin (6, 7). Strain-resisting tendons connect bones to muscles for the same reason. Acute tendon injuries are caused by severe or repetitive stresses (2, 8–10).

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, patients are more commonly unable to resume their pre-injury activities (11, 12). The mended area is also more susceptible to re-injury, especially in returning athletes (13).

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 (16, 17), physiotherapy (11) and so on. To restore normal tendon structure and function after an injury, improved therapeutic alternatives are urgently needed.

3. USE OF PRP TO TREAT TENDON INJURIES

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 patients 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 patient-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

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or high) relative to total blood, PRP delivery approach (injection or implantation). Age, kind of injury, patient activity level, treatment history, and post-recovery plans are just a few of the patient-related aspects.

PRP treatment for tendon injuries is affected by a variety of factors, but one of the most crucial is the patient's age. A person'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.

4. METHODS

Cell Expansion

Anterior cruciate ligament reconstruction was performed on three volunteers, all of whom had semitendinosus tendon biopsies. According to PI2014108, informed permission was obtained from every participant in this research. Explant culture was used to isolate tenocytes. As well as two men and one woman (mean age, 32 6 years) and two men and one woman (mean age, 32 6 years) bought them from Lonza (mean age, 39 17 years). 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 human 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).
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 3D plasma hydrogels with 4 104 cells per well as reported before. 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 3D hydrogels. 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).

5. 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-MSCs had higher directness (directionality), which is defined as displacement divided by the entire route length of the cell (P 14.017). 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. DMEM, Dulbecco modified Eagle medium, is a medium that contains Dulbecco 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. If you look 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-MSCs, 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-MSCs and skin fibroblasts (P 14.004), and a 7-fold difference between tenocytes (P 14.004). A higher amount of COL1A1 was found in skin fibroblasts than in tenocytes (P =.002) (Figure 3A).

On the other hand, BM-MSCs 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-MSCs 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 14.001), according to the data. For both anatomic cell sources, the levels of Sex expression were substantially higher in tenocytes (P 14.002). (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-MSCs. 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 14.004). Changes in matrix formation phenotype and growth factor expression induced by CM are discussed. A spindle-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 MSCs 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).

CONCLUSION

We've discussed how the type of cells used in tendon restoration can affect the biological parameters involved. When BM-MSCs and skin fibroblasts were coupled with PRP, tenogenesis was stimulated. It has been found that BM-MSCs produce less IL-8 and MCP-1, which are inflammatory proteins. Because dermal fibroblasts are more accessible, have a higher collagen I:collagen III ratio, and secrete less MCP-1 than tenocytes, they appear to be a better alternative for treating tendon lesions than BM-MSCs. These findings have implications for the design of clinical studies involving autologous cells and PRP (platelet rich plasma).

REFERENCES


