

Combination of Autologous Adipose-Derived Tissue Stromal Vascular Fraction Plus High Density Platelet-Rich Plasma or Bone Marrow Concentrates in Achilles Tendon Tears

Kristin Tate-Oliver, MD & Robert W. Alexander, MD, DMD

ABSTRACT

Background: Injury and disease of the Achilles tendon occurs not only in competitive and recreational athletes, but in the sedentary patients as well. The optimal management strategy for Achilles tendonosis and Achilles tendon tears remain controversial.¹ Whether managed surgically, or in a traditional non-surgical manner, frequently the underlying normal tendon histologic architecture and function is not completely restored.² Complications due to rupture and excess scar formation are common. This paper describes successful treatment in three patients with Achilles tendonosis and interstitial tears using with non-manipulated autologous adipose derived stem/stromal cells and high-density platelet rich plasma concentrates (or bone marrow/platelet concentrate combination).

Objectives: Introduce a safe, practical, and effective protocol for minimally invasive treatment of Achilles tendon tears using non-manipulated autologous adipose-derived tissue stromal vascular fraction with additive of high-density platelet-rich plasma or bone marrow concentrates. Explanation of use of a protocol termed "Autologous Regenerative Matrix" (ARM) is explained for guided, percutaneous placement into damaged tendinous tissues.

Materials & Methods: An automated tabletop, closed system was utilized to concentrate platelets at a high-density (defined as greater than 4 times measured baseline). This was added directly to an adipose tissue complex graft using a closed technique. A disposable, closed syringe microcannula system was employed to harvest the adipose tissue complex via standard lipoaspiration techniques. Ultrasound guidance was employed for accurate placement of the tissue graft with additive into injured site of Achilles tendon. Physician examination, visual analog pain scale, and serial high definition ultrasonography was performed to document tissue disorder and responses to therapy.

Findings: Initially all patients experienced a mild to moderate increase in discomfort for 3-4 days, following which each patient had improvement in pain level or returned to pre-treatment baseline level. Significant improvement in reported pain levels began within 3-4 weeks, and steadily improved in all cases. At 12 weeks, all patients returned to full activity without pain or dysfunction. At the time of

this report, 3-4 years have elapsed, with all patients maintaining full resolution of symptoms and no re-occurrence. Repeat ultrasound studies of at least 12 months post-treatment showed restoration of normal tendon structure without excess fibrosis, normal tendon configuration, including size and palpation tenderness. Objective metrics and findings lagged behind the patient's subjective improvement in each example.

Discussion/Conclusions: The percutaneous use of a combination of autologous adipose tissue complex (inclusive of its heterogeneous cell populations and native bioactive scaffold) and either HD-PRP or HD-PRP/BMAC combination appear as promising options for non-surgical management of Achilles tendonosis and partial thickness interstitial tears. The method is safe, very well tolerated by patients, uses readily available autologous sources, and utilizes non-manipulated autologous tissues. Treatment with ARM protocol is improved with use of high-definition ultrasound to ensure target correct placement. This case series is intended to introduce in this treatment option, and encourage the development of a larger trial in the future.

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KEYWORDS: achilles tendon tears, adipose stem/stromal cells, autologous adipose stromal vascular fraction, autologous regenerative matrix, biocellular therapy, mesenchymal/pericyte cells, platelet-rich plasma.

Background & Introduction

The Achilles tendon is the largest and strongest tendon in the human body, and a common site for injury and disease. It is well accepted that the "triad" comprised of Achilles tendon pain, activity reduction, and site swelling should best be termed tendinopathy vs. tendonitis. This is confirmed by histological studies demonstrating tissue degeneration vs. acute inflammation.⁴ The exact etiology remains elusive with no benchmark study identifying a direct causal

relationship, but known contributors include overuse, foot mal-alignment, vascular diseases, neuropathy, and rheumatologic diseases may place a patient at an increased risk.⁵ Damaged, weakened, inelastic fibrotic tissue (including excess scar formation) predisposes the Achilles tendon to increased risk for development of recurrent partial or full thickness tears.³

The incidence of Achilles tendon tears in the general population is 7 per 100,000.^{6,7} The most common mechanisms of injury include sudden forced plantar flexion of the foot, unexpected dorsiflexion of the foot, and violent dorsiflexion of a plantar flexed foot. Other mechanisms include direct trauma and, less frequently, attrition of the tendon as a result of longstanding peritendonitis (with or without tendonosis).⁷⁻¹⁰ Over 80 percent of Achilles tendon tears occur during recreational sports. Approximately 10 percent of patients who sustain an Achilles tendon rupture report pre-existing Achilles tendon disease.¹¹

Observational data suggest that competitive athletes have a lifetime incidence of Achilles tendinopathy of approximately 24 percent, with 18 percent of those sustained by athletes younger than 45 years.¹² Tendon rupture incidence occurs in 8.3 percent of reported injuries. Among competitive runners, the lifetime incidence of Achilles tendinopathy may be as high as 40 to 50 percent. Other populations at risk for Achilles tendonosis include those who are poorly conditioned, those of advanced age, those who have been using fluoroquinolone antibiotics, those who have been treated with corticosteroids, and those who overexert themselves.^{3, 6-7, 13}

Review of the literature reports common treatment options of Achilles tendonosis to include rest, activity modification, NSAIDs, local steroid injections, injection of local sclerosing agents, physical therapy, surgical debridement and/or grafting, extracorporeal shock wave therapy, eccentric muscle training, bracing, orthotics, percutaneous tenotomy, Focused Aspiration of Scar Tissue (FAST procedure), and use of biological platelet rich plasma injections. Surgical management is typically reserved for those patients who do not respond to conservative treatment regimens.^{8, 9, 12-18}

The literature regarding the treatment of Achilles tendon rupture remains controversial. The goals of treatment of a ruptured Achilles tendon are to restore *length and*

tension as a means of returning the structure's *strength and function*. Satisfactory outcome can be achieved with operative and traditional non-operative treatment, but complications such as re-rupture and poor post-treatment functional ability, exist with either approach.^{3, 11-13} Traditional non-operative modalities used in Achilles tendon healing consist of various types of cast immobilization and physical therapy. There is little agreement in the literature on the optimal method of cast immobilization, and even with aggressive rehabilitation, many patients are unable to reach their maximal level of function.^{16, 19-21} In the event that a "gap" in the Achilles tendon is healed with scar fibrosis, there results an inherent residual weakness of push off strength and reduced power. This loss of tendon integrity and scarring is commonly accompanied with high incidence of recurrent injury.¹¹

Various surgical techniques have been reported regarding the treatment of Achilles tendon tears. These techniques include open repair, with and without tendon tissue graft augmentation. Open surgical approaches appear to have a slightly lower risk of re-rupture than traditional non-operative treatments, however, the risks of complication from wound infection, delayed wound healing, donor site morbidities, thromboembolism, and Sural Nerve damage are high.²² The Cochrane Library reviewed surgical interventions in 2010 reporting that open surgical treatment compared with non-surgical treatment (6 studies, 502 participants) was associated with a lower risk of re-rupture, but a higher risk of other complications such as infection, adhesions and disturbed skin sensibility (numbness and tingling). Percutaneous surgical repair (involving stab incisions through which the repair suture is passed through without direct exposure of the tendon) compared with open repair (4 studies, 174 participants) was associated with a lower risk of infection, but did not address long-term success or incidence of recurrence. There exists insufficient and inconclusive data on function and sporting activities when comparing surgical to traditional non-operative interventions.²³

We herein report a safe and efficacious percutaneous non-surgical alternative using ultrasound guided to treat Achilles tendonosis and non-retracted Achilles tendon tears with more than 7 years of experience. This protocol and approach is termed "Autologous Regenerative Matrix" (ARM). ARM consists of a combination of autologous adipose-derived *tissue* stromal vascular

fraction (AD-tSVF) with additive of high-density platelet-rich plasma (HD PRP – defined as a *minimum* of 4-6X measured baseline concentration). In some cases, the AD-tSVF may be combined with bone marrow concentrate (BMAC) as the additive biologic product. This protocol and technique will be presented in more detail later in this article.

When tendons are injured and attempt to heal, normal tendon histology is often not completely restored, and neither is complete return to strength and function. Tendons heal with an intervening layer of scar tissue whose component and mechanical properties are often inferior to native tendon tissues. This effectively results in failure of both traditional non-operative and operative repair of torn tendon. It likewise may make them more susceptible to adhesion formation due to undesirable scar tissue formation. The inability of the tendon to self-repair, and the relative inefficiency of current treatment regimens, suggest that identifying alternative strategies should become a priority.^{22, 24-27} Therapies that can augment regeneration of normal tendon, while limiting the amount of poorly oriented excessive scar tissue that is formed in response to injury, may improve clinical outcomes and durability.^{2, 28-34}

One such alternative is the use of autologous stem/stromal cells within AD-tSVF to favor repair of damage or disease, either through direct application, or in conjunction with its native “bioactive” 3-D scaffolding (offering paracrine secretory effects of the extracellular matrix.^{22, 35} Access to multipotent stem/stromal cells and adipose-derived bioactive native matrix, is readily available with use of adipose-derived tissue stromal vascular fraction (AD-tSVF) provided by microcannula closed syringe lipoaspiration harvest. This has shown the capability to provide effective pro-inflammatory and immune modulation to injury sites.^{36, 37} The microenvironment appears to be directly influenced, or directed, by both the cellular contribution and bioactive paracrine signaling. These components may differentiate (cellular) or serve as important paracrine (chemical) functions to a site of injury or inflammation. To that end, the elements provide, or influence, a variety of different cell types including osteogenic, chondrogenic, and tenocytic lines while favoring healing mechanisms.³⁸ If the microscopic architecture of damaged tendon could be restored to normal, not only would pain and function be expected to improve, but risk of tear or re-rupture significantly reduced.

The diverse mononuclear cell population existing within the tSVF has proven to be very heterogeneous, making identification of the single cell types of greatest importance impossible to determine at this time. Both pericyte/endothelial cells and mesenchymal stem cells (MSCs), are believed to represent an important multipotent cell population residing in all perivascular tissue microenvironments. The tissues include adipose tSVF and bone marrow. Due to the adipose tissue complex representing the *largest microvascular organ* in the body, capable of providing much higher numbers of these cells (such as mesenchymal and pericyte populations) are in much higher concentrations within this tissue site. When these cells are adherent to other cells or extracellular matrix (ECM) within a tissue microenvironment (niche), they are capable of active proliferation and differentiation (including transdifferentiation) into various target tissue types, including tenocytes and myoblasts.³⁹

For many years, bone marrow derived stem cells (BM-SC) were considered the primary source of stem cells for tissue engineering applications. Since discovery of the significantly higher concentration differences and the similar *In Vitro* differential capabilities available in adipose tissues, extensive research is now devoted to the examination of AD-tSVF and AD-cSVF, as offering great potential clinical applications in regenerative medicine.⁴⁰

It has been shown that AD-tSVF possesses remarkably similar components and capabilities of pro-inflammatory wound healing, immuno-privileged abilities, and biocellular differentiation potentials as reported for marrow. Further, ability to harvest AD-tSVF via closed syringe, microcannula lipoaspiration techniques have proven of advantage to access these cells and tissues. Adipose tissue access has multiple advantages over marrow due to its extensive non-hematopoietic multipotent cellular and bioactive matrix. Access to tSVF is available by well-established techniques featuring advantages of requiring a less invasive procedure using a plentiful resource, supported by well documented safety and efficacy, using a much less expensive harvesting technique.^{41, 42} In addition, AD-tSVF has been well documented to possess significantly higher concentrations of mesenchymal stem cells compared to bone marrow (1000-2500 times in adipose versus marrow), making adipose-derived stem/stromal cells (including mesenchymal cells and the bioactive native matrix) an attractive option for many applications in the field of regenerative medicine.^{43, 44}

For over 25 years, cosmetic-plastic and reconstructive techniques have employed autologous fat grafting techniques extensively in dealing with structural defects, wound healing, tissue augmentation and bone regenerative applications, with extensively documented safety and efficacy records.⁴³ Evidence has shown that both the mature and progenitor cells found in the adipose tissue stromal vascular fraction (tSVF) and the native bioactive, extracellular matrix (ECM) of adipose, are important contributors in tissue healing.⁴⁶

For many years, it was believed that adipose tissues were relatively static pertaining to cell replacement, only changing in the volumes of lipid stored within the cytoplasm of mature adipocytes. We have since learned that the natural homeostatic mechanisms lead to total replacement over 5-10 year period.⁴⁷ It is understood that the natural senescence process of mature adipocytes, result in release of important growth factors and signal proteins. These, in turn, stimulate their attached progenitor cells (near terminally differentiated pre-adipocytes via cell-to-cell and paracrine signaling to activate and differentiate into a metabolic mature phenotype. On that basis, physical (mechanical) stimulation, tissue oxygenation, cellular interactions, and paracrine activation of a variety of progenitor cells attached within the SVF (including an extensive perivascular and extracellular matrix) are considered inherently important.⁴⁸⁻⁵⁰

These factors are intrinsically responsible for activation, proliferation, and differentiation within tissues and appear to encourage site healing and regenerative outcomes.⁵¹ For musculoskeletal applications AD-cSVF cells have been shown, *in Vitro*, to differentiate into tendon, ligament, skeletal muscle, bone, nerve and cartilage.⁵² When AD-tSVF is introduced into non-adipose mesogenic-derived tissues (as listed above), events mimicking the homeostatic mechanisms are initiated. This includes mature adipocytes degeneration, but permitting release of important growth factors, signal proteins, and cytokines/chemokines to contribute within the recipient tissue in a “site-specific” fashion. This allows the cellular and chemical interactions of the native microenvironment to favor regeneration of the damaged tissue with the niche-correct cell line.⁵³

The authors’ experiences support the hypothesis that successful autologous biologic adipose graft and additives are optimally effective in musculoskeletal applications. In order to accomplish this goal, precise placement of

the 3 major components plays an important interactive role to: (1). Provide *living bioscaffold* native to adipose tissues; (2). Provide plentiful *quantities of undifferentiated, heterogeneous cellular populations*; and, (3). To deliver *highly concentrated key growth factors/signaling proteins* important to wound healing. Each of these component parts is considered equally important.

The adipose tissue complex (ATC), mixed with either high-density platelet concentrates (HD-PRP) or bone marrow concentrates (BMAC), safely and effectively provides all of these components. There exists a *direct* correlation between the achieved platelet concentration and the measurable release of higher concentrations of multiple growth factors, cytokines/chemokines derived from platelet degranulation. In addition, the higher platelet concentrations achieved result in much higher rates of proliferation and migration effects on key mesenchymal and perivascular cells. It is well known that “all platelet concentrate systems” are NOT equal, and that it is important to achieve *at least* a 4X baseline (or greater) to reach maximum potentials and results. (See Figure 1.)

When this AD-tSVF and HD-PRP combination is accurately introduced into a damaged or degenerating site, provision of needed autologous wound scaffold, healing elements, and diverse cellular resources effectively permit full responses to encourage full site-specific healing or regeneration of tissues is the result.⁵⁴ Use of a

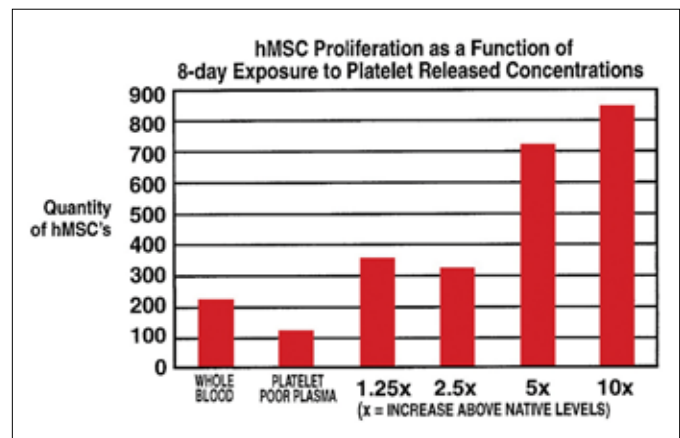


Figure 1. Direct relationship of concentration levels of platelet concentrates (over measured baselines) and mesenchymal stem cell proliferation and migration to areas of injury or inflammation.

Adapted from Haynesworth SE, Bruder SP, et al. Mitogenic stimulation of human mesenchymal stem cells by PRP suggests a mechanism for enhancement of bone repair. Presented at 48th Orthopaedic Research Society Meeting, Dallas, TX, 2002.

true autologous tissue graft, employing non-manipulated AD-tSVF and HD-PRP concentrates has proven of value in a wide variety of cosmetic-plastic surgery and musculoskeletal applications, including tendonous tissues as reported in this paper. To that end, it is convenient to refer to the combination of non-manipulated biocellular components as “Autologous Regenerative Matrix (ARM)”.⁵⁵

Although there exists means and methods to isolate and concentrate AD-MSC and other subsets of cells from adipose tissue stroma, these technologies involve either mechanical or chemical digestion and such manipulation of human progenitor cells is not currently permitted for clinical use in the United States.⁴⁸ One author (RWA) has successfully used non-manipulated autologous adipose grafts with autologous platelet concentrates extensively for more than 20 years in the aesthetic, reconstructive, and chronic wound surgical applications with great success. The enhancement of grafting with the use of HD PRP concentrates as an effective additive to promote tissue acceptance and healing has been well documented and reported.⁵⁶⁻⁵⁸

Materials & Methods

Patients presenting with tendonosis or interstitial tears of less than 3 cm are routinely treated with ultrasound guidance for accurate placement of autologous adipose graft (AD-tSVF) plus additive of HD-PRP. In this series, one patient with an interstitial tear greater than 3 cm was treated with placement of autologous fat graft (AD-tSVF), plus used of combined additive (bone marrow and high-density platelet concentrate).

In the cases of use of the additive, acquisition of HD-PRP was provided via routine venipuncture techniques to provide, a simple peripheral blood sample. A blood draw of 54 cc was performed, and the blood processed at bedside via the closed, automated Harvest SmartPREP II System (Harvest Technology, Plymouth, MA) following manufacturing guidelines. Bidirectional centrifugation (at approximately 1000 g force) and patented shelf resulting in approximately 8-10 cc of HD-PRP concentrate. This technology has well documented, extensively delivered platelet concentrations of >4-6 times measured actual patient baselines.

Bone marrow aspirate concentrate (BMAC), a standard approach to the posterior-superior iliac crest including examination and guidance with ultrasound was performed. Following manufacturer's protocol, bone marrow concentrate was created utilizing the *same* SmartPREP II centrifugation system and sterile BMAC kit supplied by Harvest Technologies (Plymouth, MA) for that specific purpose. In the specific case presented in this paper, additional peripheral blood draw of 28cc was obtained and used for creation of HD-PRP. The bone marrow aspirate and the HD-PRP were subsequently *combined* and re-processed in the Harvest SmartPREP II closed centrifuge to provide 7 cc of concentrate of bone marrow aspirate plus the HD-PRP.

Acquisition of quality autologous adipose graft (ATC) via lipoaspiration techniques follows a simple standard sterile protocol. Papers are available to clearly outline the disposable system and techniques which are well within the non-surgeon capabilities.

This technique of small volume lipoaspiration was used to provide approximately 20 cc of adipose tissue complex, using the patented disposable Tulip (Tulip Medical, San Diego, CA) closed syringe microcannula lipoaspiration system. (See *Figures 2a-d*.) Selection of donor site for adipose graft harvest was based on individual patient deposit locations. This donor areas most commonly used were the lower abdomen-flank area (male and female) or lateral thigh-buttock area (females). (See *Figures 3a & 3b*.)

A standard (2.1 mm X 10 cm) multiport infiltrator blunt cannula was employed to inject approximately 15-20 cc of 0.05% lidocaine with 1:1million epinephrine into the subcutaneous fat layer within a pre-defined area. By use of a repeatable fan-like pattern, dilute local anesthesia was distributed in the adipose layer to provide a liquid “vehicle” for small adipose fragments to attain a suspension state. This distribution followed by passage of the cannula without vacuum pressure in the same pattern, which is termed “pre-tunneling” within the area to further distribute local fluid, but most importantly assist in the gentle mobilization of adipose graft tissue. Lipo harvest was performed via a 20 cc luer Monoject syringe with an offset Carraway harvester (three ports, 2.1 mm x 8 cm), and low vacuum pressure, until acquisition of approximately 18-20 cc total volume lipoaspirate (including fluid, autologous fat graft (Adipocytes and AD-tSVF), and extracellular free lipids) was attained.



Figure 2a. Disposable closed syringe microcannula system.
Tulip Medical™, San Diego, CA.



Figure 2d. External "lock" to apply vacuum.
Tulip Medical™, San Diego, CA.



Figure 2b. Disposable cannula set for closed microaspiration system. Top: Internal "Lock" Option To Apply Vacuum. Middle: 2.1 mm (OD) X 12 cm Multiport Infiltrator Cannula (Distribute Local Fluid Carrier). 2.1 mm X 10 cm Offset Carraway Harvester. 1.25 mm X 6 cm Single Port Injector Cannula. Bottom: Clear Luer-to-Luer (Anaerobic Transfer) Transfer.
Tulip Medical™, San Diego, CA.

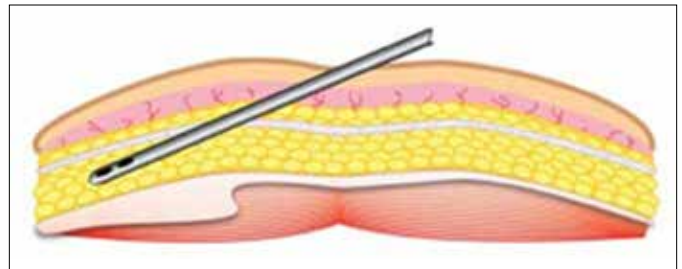


Figure 3b. Diagram of ideal depth of microcannula aspiration from abdomen. White line representing scarp's fascia.

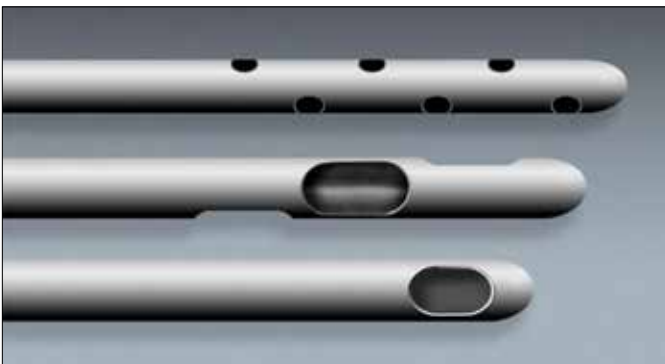


Figure 2c. Close up of microcannula tips. Top: Multiport Injection Cannula. Middle: Offset Carraway Harvester Cannula. Bottom: Single Port Injector (Blunt) Cannula.
Tulip Medical™, San Diego, CA.

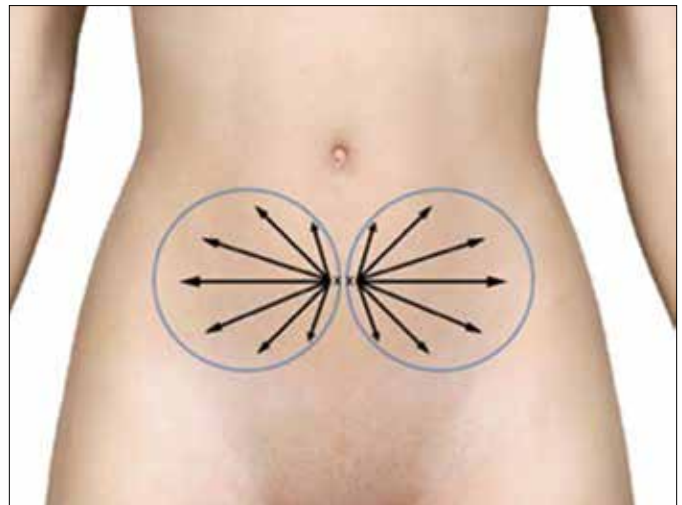


Figure 3a. Diagrammatic example of lower abdomen harvest areas. Arrows represent the fan-shaped pattern useful for harvesting adipose grafts.

(See Figure 4.) Gravity decantation was utilized to provide a density gradient separation to permit separation of the unwanted lower (infranatant) fluid layer (containing debris, blood cells, and a few nucleated cells dislodged during aspiration). (See Figure 5.) The unwanted, clear free lipid layer (supranatant layer) was carefully avoided during the preparation of the AD-tSVF prior to mixing the graft with the select additives (ARM). Inadvertent inclusion of the free lipid layer may result in unwanted inflammatory stimulation. Sterile closed, clear “anaerobic”, transfers (luer-to-luer) are used to withdraw and dispose of the infranatant layer, and permit graft isolation leaving the lipid layer in the harvesting syringe. (See Figure 6.)

Use of centrifugation has gained recognition in provision of improved layer separation, compression of the graft (with less residual fluid), and minimize exposure of the graft to residual local anesthetic. Centrifugation is currently available using the same SmartPRep system using tissue processing syringes with a separating disk to more effectively remove the free lipid layer. (*AdiPRep, Harvest Technologies, Plymouth, MA*). (See Figures 7a-b.) This step is commonly used in cosmetic-plastic surgical applications, but was not available at the time of the patient series treatments.

The resulting adipose autograft provided approximately 10-12 cc volume of quality adipose graft, a portion of which was mixed via the closed luer-to-luer connections with approximately the same volume of HD-PRP. This constitutes the component parts of the therapeutic portion of the ARM protocol. (See Figure 8.)

Using ultrasound guidance, the ARM components can be precisely introduced into the target tissue site (patient’s injured Achilles tendon) in the areas of documented abnormality. Prior to percutaneous injections, each site of injection was surgically prepped, isolated, and often followed by topical ethyl chloride topical anesthesia for patient comfort prior to injection.

Ultrasound guidance was employed throughout the procedure to ensure correct percutaneous placement of an 18-gage needle into the site of tendonosis/tear. The isolated treatment areas were injected with 1 cc of 1% lidocaine to provide patient comfort, following which the syringe was changed to attach the autologous ARM mixture for guided introduction of between 1–5 cc directly in the damaged tissues. The ratios of the mixture

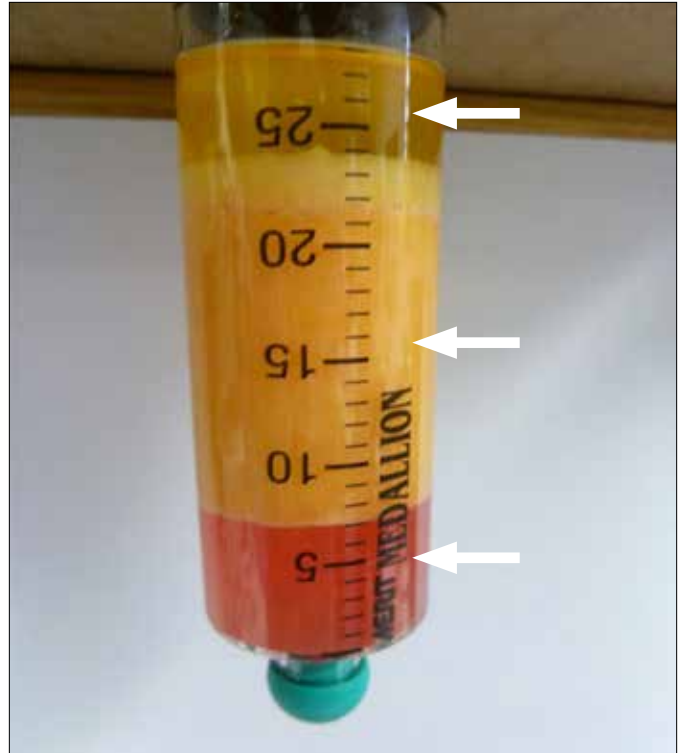


Figure 4. Three gradient separation layers in lipospirates. Top arrow: supranatant free lipid layer. Middle arrow: adipose tissue graft (tSVF). Bottom arrow: infranatant fluid & debris layer.



Figure 5. Gravity decantation for layer separation (4 minutes) on luer base.
Tulip Medical™, San Diego, CA.



Figure 6. Closed luer-to-luer transfer to remove graft from unwanted free lipid layer (top syringe).
Luer-to-luer transfer – Tulip Medical™, San Diego, CA. Shown in Tissue Processing Syringe – Harvest-Terumo, Plymouth, MA.



Figure 7a. AdiPrep centrifuge. 4 minute cycle at approximately 1000 g force, showing tissue processing syringe (with disk) on right. Harvest Technologies, Plymouth, MA.

used in musculoskeletal applications optimally range from 50:50 to 70% Graft to 30% HD PRP (by volume). In these reported case examples, the 50% mix ratio was employed (approximately 1 cc of lipoaspirate per 1 cc of additive HD-PRP per centimeter injured tissue). In all cases, we recommend a “needling” or “peppering” of the teno-osseous junctions if involved in the pathology. Sterile adhesive dressings were applied and the ankle was kinesioted for support for 3 days.

Patients are instructed to keep moving the extremity, and to gently ambulate as tolerated. Early limited ambulation is encouraged to provide mechanical stimuli that are considered important for proper wound healing with tendon architectural alignment. At one week, patients are started on a home exercise therapy consisting of eccentric stretching, and allowed to return to light activity as tolerated. All patients were interval tracked at 2 weeks, 4 weeks, 8 weeks, and 12 weeks. Patients were re-evaluated with high-definition ultrasonography of the treated areas during their next available appointments, typically at 8-12 week post-treatment.

We report a series of patient cases where the ARM protocol was utilized in the successful treatment of chronic Achilles tendinopathy with high-grade partial thickness Achilles tendon tears.

CASE PRESENTATION 1:

A 61 year-old Caucasian female homemaker presented with one-year history of severe pain in the posterior right ankle with marked limitation of normal daily activity. No report of known specific traumatic event was identified. She experienced gradual onset of symptoms with slowly increasing pain and distortion (thickening) of the insertional Achilles area. On initial evaluation, the pain was described as sharp with report of severe daily pain that limited her normal activities, and was walking with a severe limp. Previous evaluation by her primary physician as well as a local orthopedic surgeon resulted in a diagnosis of Achilles tendonosis and longitudinal partial thickness tear from mid-substance to insertion.

Prescription of rest, physical therapy, and NSAIDs were offered initially, followed by corticosteroid injections to the Achilles tendon prior to her initial visit. Following steroid injections, placement of night splint resulted in essentially no clinical improvement. During her initial visit, she was wearing a CAM-type walking-boot that she



Figure 7b. Post-centrifugation (4 minutes @ 1000g force) layer separation (density gradient). Top: free lipid layer & separating disk. Middle: adipose tSVF compressed after centrifugation. Bottom: infranatant liquid & debris layer.



Figure 8. Top: High-density platelet-rich plasma (6 x measured baseline). **Bottom:** adipose-derived tissue stromal vascular fraction (following removal of infranatant fluid and debris, and avoidance of free lipid layer from supranatant layer).

had been wearing for approximately 12 weeks prior to consultation. She had previous x-rays and MRI prior to her first visit. X-rays were unremarkable, and the MRI demonstrated severe insertional tendonosis with presence of two longitudinal interstitial tears of the Achilles tendon extending from mid-substance to insertion. No full thickness tears were identified.

The patient had a pertinent history of psoriasis and psoriatic arthritis and was taking Remicaide. Other medications for hypertension and hyperlipidemia were not considered relative.

Initial physical examination of her right ankle confirmed a grossly enlarged Achilles tendon that was extremely palpation tender, from mid-substance to insertion. Increased pain was elicited with active plantar flexion against resistance, and was unable to elevate on her right foot toes with full weight bearing. This was not the case on the contralateral side. Diagnostic ultrasound demonstrated severe tendonosis and longitudinal tears of the Achilles tendon extending from mid-substance (See Figure 9.) to insertion. (See Figure 10.)

Patient consent for ARM protocol treatment (using HD-PRP additive to autologous adipose graft) of the involved Achilles tendon was obtained, to include alternative therapeutic options (including no treatment). Adipose graft was harvested from lower abdomen, followed by addition of HD-PRP (50:50 concentration by volume) to treat the patient's right Achilles tendon. Specific areas treated were identified and guided by use of high-definition ultrasound, and included sites of interstitial tear, teno-osseous insertion, and peri-tendonous sheath area.

The patient was re-evaluated at 2, 4 weeks, and ultrasound evaluation at 8 weeks after treatment. Right ankle pain was improved from a 9/10 on a visual analog scale to a 4/10. She reported the ability to walk one mile with minimal soreness, and was performing all her daily activities without discomfort. Examination showed resolution of limp, and significantly less tenderness to palpation. The observational swelling remained relatively unchanged at the 8 week evaluation. Diagnostic ultrasound demonstrated marked improvement in the heterogeneous tendon changes noted on first exam, including improvement of the interstitial tears at mid-substance. (See Figure 11.)

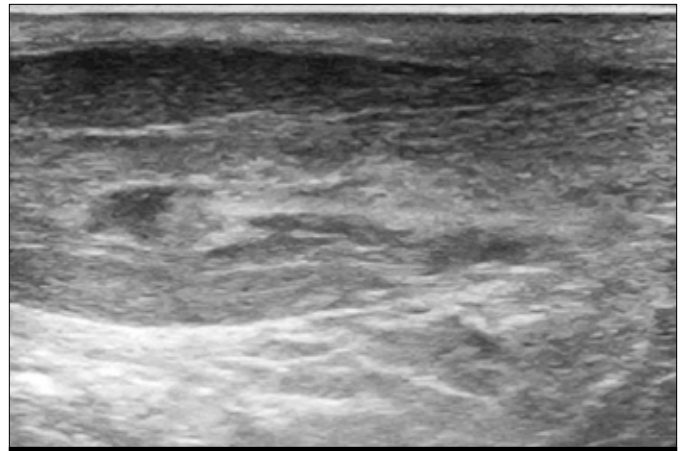


Figure 9. Case 1: Initial presentation US of right Achilles tendon. Note: Severe tendonosis and longitudinal tears at mid-substance and extending distally.

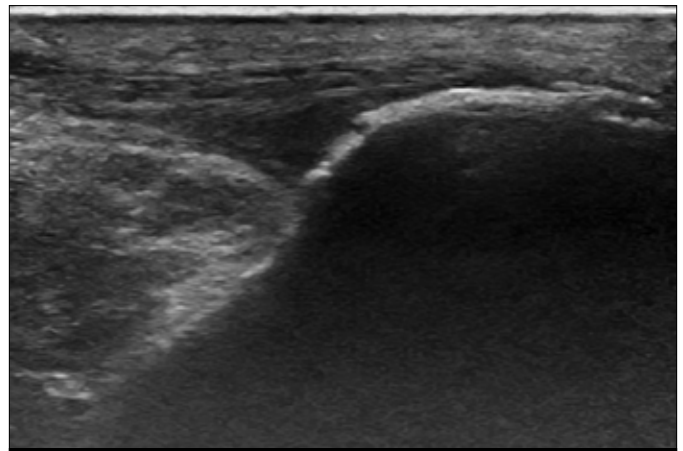


Figure 10. Case 1: Initial presentation US of right Achilles tendon showing severe tendonosis and longitudinal tears noted at insertion.

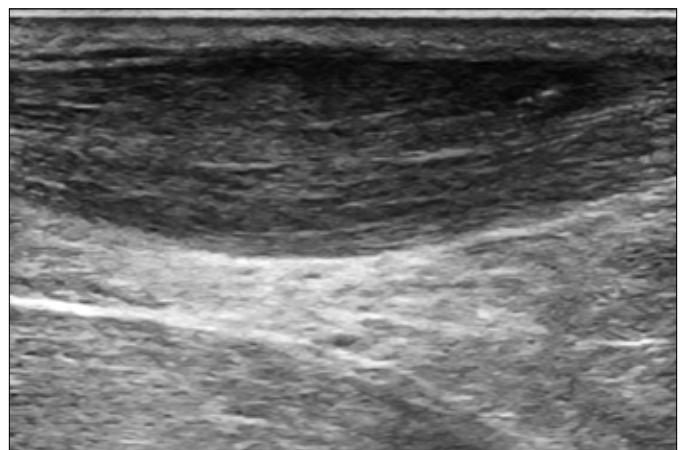


Figure 11. Case 1: eight weeks post-biocellular ARM treatment. Longitudinal tears improved, but continued marked tendonosis noted mid-substance.

A second stage ARM therapy was recommended, and performed at the 8-week visit. The same treatment protocol was employed as performed in the initial graft.

Re-evaluation at 24 weeks following the **initial** ARM treatment included examination and an ultrasound evaluation. Right ankle pain improved from the original 9/10 pre-treatment on a visual analog scale to a 1/10. She reported return to full function, including the ability to walk two miles with no pain. Physical exam confirmed resolution of the previous tenderness to palpation and improvement of tendon thickness. The observed swelling was markedly reduced, with only small areas of non-tender residual thickness to firm palpation noted. Diagnostic ultrasound demonstrated marked improvement of the heterogeneous changes noted pre-treatment exam, with all tears fully resolved. (See Figure 12.) The tendon did remain mildly to moderately thickened at mid-substance.

Patient evaluation (for a different issue) at approximately one year post-treatment, and again at 3 years, revealed continued lack of pain in the right ankle, with pain level recorded as 0/10. Ultrasound demonstrated the Achilles tendon and sheath to be of normal echogenicity, and the previous tears were no longer evident. The thickness of the tendon had improved by an additional 40%, resulting in minimally increased residual tendon thickness, and resolution of all subjective and objective findings. Patient was last seen at 18 month following the original ARM therapy, with Achilles and ankle remaining pain free with no functional limitations or activity restrictions. Ultrasound evaluation showed Achilles tendon to have a small degree of intra-substance heterogeneous changes at insertion and minimal residual mid-substance swelling but otherwise appeared normal. (See Figure 13.)

CASE PRESENTATION 2:

60 year-old retired male presented with 7-month history of pain in the posterior left ankle. Injury was reported several months prior to consultation with sudden onset of severe pain following a slipping fall while fly-fishing. This event was aggravated in the interim playing competitive racquetball. The pain was described as sharp, severe daily pain, limited function in work and activities, and was walking with a marked limp. Prior to evaluation, his primary care physician referred him to an orthopedic surgeon who diagnosed a high-grade partial thickness tear (>70% of thickness) of the left Achilles tendon. He was recommended to undergo open surgical repair with either split tendon or cadaveric grafting.

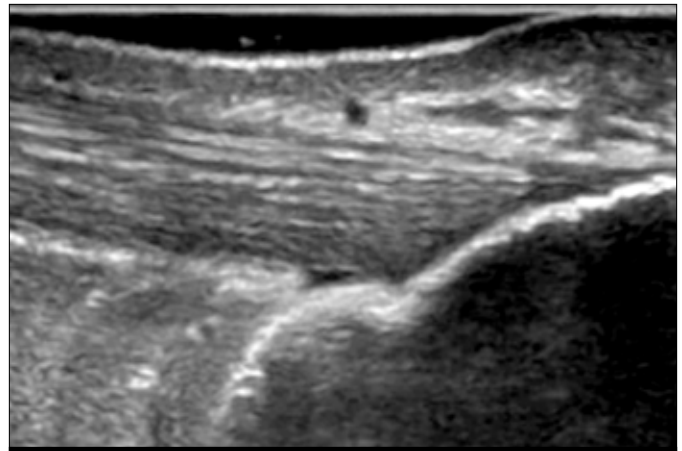


Figure 12. Case 1: Two months after biocellular ARM placement. Right Achilles tendon to insertion demonstrating marked improvement of previous heterogeneous changes and resolution of previous tears.

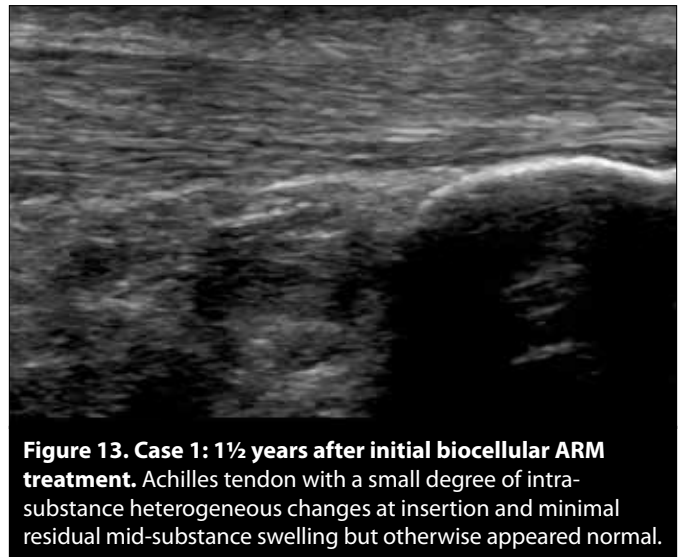


Figure 13. Case 1: 1½ years after initial biocellular ARM treatment. Achilles tendon with a small degree of intra-substance heterogeneous changes at insertion and minimal residual mid-substance swelling but otherwise appeared normal.

Prescription of rest, NSAIDs, physical therapy, and immobilization in a CAM unhinged walker boot for over 4 weeks. X-rays and MRI prior to our initial visit confirmed the diagnoses via MRI, showing a high-grade partial thickness interstitial tear (>70% of thickness). The patient's relative medical history was considered unremarkable, and was taking no prescription medications.

Evaluation of the left ankle area revealed a grossly enlarged Achilles tendon on inspection, with extreme tenderness to palpation proximal to insertion. Examinations revealed a palpable defect (>2 cm in length) with its distal-most aspect being approximately 2.5 cm *proximal* to insertion. Pain upon actively plantar flexion against resistance was

severe, and patient unable to raise-up on his left foot toes under full weight bearing load (due to weakness and pain). A Negative Thompson's test was found. Diagnostic ultrasound demonstrated severe tendonosis and presence of a high grade longitudinal tear of the Achilles tendon (See Figure 14.) approximately 2.5 cm proximal to insertion, extending to the musculo-tendinous junction of the soleus (total size of 6.3 cm x 0.8-1.1cm). In addition, dynamic ultrasound examination demonstrated muscle defects not previously identified on standard MRI examination. These sites were treated with the ARM protocol at the same time as the Achilles treatment.

Consent for use of ARM was obtained, including discussion of alternatives to include no treatment. Due to size and extent of longitudinal intra-substance tear, we elected to use combination of autologous adipose graft with a combination mix of both HD-PRP and BMAC in the standard therapeutic modality. Ultrasound guidance was utilized to clearly identify the areas of muscle defects, insertional tear and severe tendonosis.

Clinical follow up was performed bi-weekly for 4 months. Full physical and ultrasonic re-evaluation was performed at 24 weeks post-procedure. Patient's left ankle pain was improved from a 10/10 on a visual analog scale to a 0/10. At this visit the patient reported no pain, full return of normal activities, return to full exercise capabilities (including bi-weekly racquetball). No limitations were noted relative to pain or loss of function. Exam confirmed resolution of limp and tendon non-tender to firm palpation. Observational swelling was markedly improved with resolution of the palpable defect. Diagnostic ultrasound documented full resolution of the heterogeneous changes in the muscular defects and longitudinal tear areas. (See Figure 15.) No second ARM treatment was performed. Post-treatment follow up at >3 years confirmed that injured ankle remains pain free, fully functional, and has no restrictions of activities.

CASE PRESENTATION #3

34 year-old male presented with a 7-month history of pain in the posterior left ankle. Sudden onset of pain was reported while running with an accompanying "pop" and "tearing sensation" in the posterior left ankle. Sharp pain upon ambulation was followed by a dull ache following rest. Initial evaluation confirmed constant pain that significantly limited his work and daily activities plus an accompanying limp.

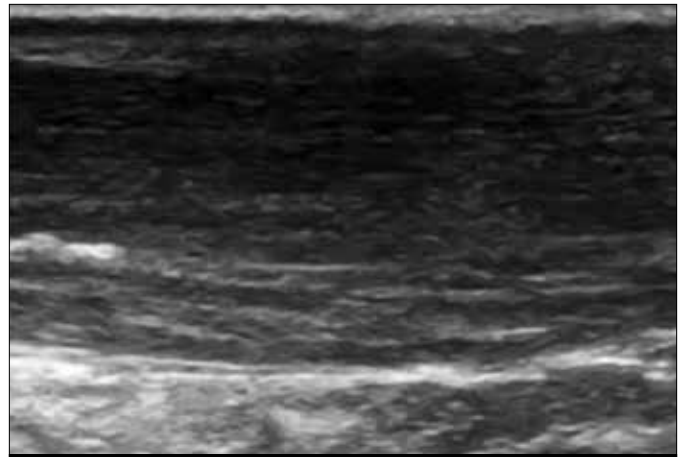


Figure 14. Case 2: Initial presentation mid-substance Achilles with severe tendonosis and longitudinal tears noted mid-substance.

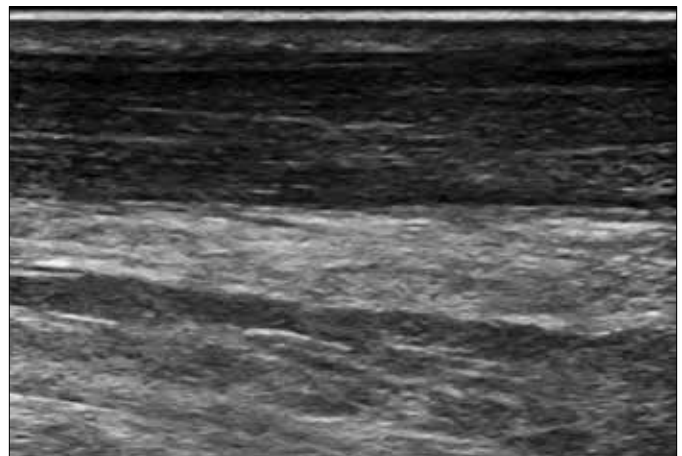


Figure 15. Case 2: Six months following initial biocellular ARM treatment. Note: markedly improved heterogeneity and thickness.

Initial evaluation by his primary care physician included exam and X-rays, which were reported as negative. He was prescribed rest, physical therapy and NSAIDs without relief. The patient had no significant pertinent medical history and was taking no daily medications (except for the NSAIDs).

Initial evaluation of left ankle revealed a painful, grossly enlarged Achilles tendon. Injury extended from insertion of Soleus myotendinous junction to approximately 8 cm proximal to the Achilles tendon bony insertion. Increased pain upon active plantar flexion against resistance was noted, and patient was not able to raise-up on his left toes (full weight bearing). Contralateral evaluation was normal and without pain or limitation.

Diagnostic ultrasound demonstrated severe tendinosis and interstitial longitudinal tear of the Achilles tendon measuring 1.36 by 0.29 cm at mid-substance (See Figure 16.) with small extensions distally to insertion, and proximally approaching the Soleus myotendinous area.

Informed consent for use of ARM protocol was obtained, including discussion of all treatment alternatives. Routine surgical preparation for isolation of the treatment site was provided, and ultrasound used for identification and guidance to specific areas of insertional tear and severe tendinosis. Autologous adipose graft was harvested from the flank area and was combined with HD-PRP as additive at a ratio of 50:50 by volume.

The patient was monitored at 2 and 4 weeks, and returned to clinic at 8 weeks for examination and post-treatment ultrasound evaluation. Left ankle pain was improved from a 9/10 on a visual analog scale to a 2/10. He reported the ability to walk increased distances without pain or discomfort. He was performing all his normal daily functions without pain or functional abnormality. Exam showed resolution of the previous limp, near non-tender Achilles tendon to palpation except in the retro-calcaneal area. The observational swelling was not substantially changed compared to pre-treatment at that point. Diagnostic ultrasound demonstrated marked improvement in all changes noted on initial exam, and marked improvement of the interstitial tear. (See Figure 17.) A moderate retro-calcaneal bursal hypoechoic fluid collection was noted at this time and may have accounted for the minimal residual pain. No further treatment was provided for that finding.

The patient was asked to return at 24 weeks post-treatment for re-evaluation, but he reported resolution of the residual pain and chose to not keep his follow-up appointment. Patient contact at the 24-week interval reported return the ability to run distances of 4 miles without limitation, and he felt no further treatment was necessary. The patient was seen at one 1 year later (for an unrelated complaint of shoulder pain). At that time a repeat ultrasound examination of the right Achilles tendon was obtained. Previous intra-substance tear and tendinosis was documented as fully resolved, with complete return to all activities without pain or functional limitation. (See Figure 18.)

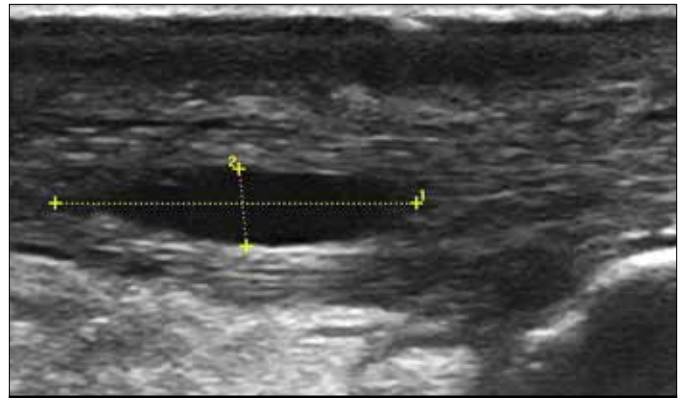


Figure 16. Case 3: Initial presentation demonstrating large interstitial tear and marked thickening and tendinosis of right achilles tendon mid-substance.

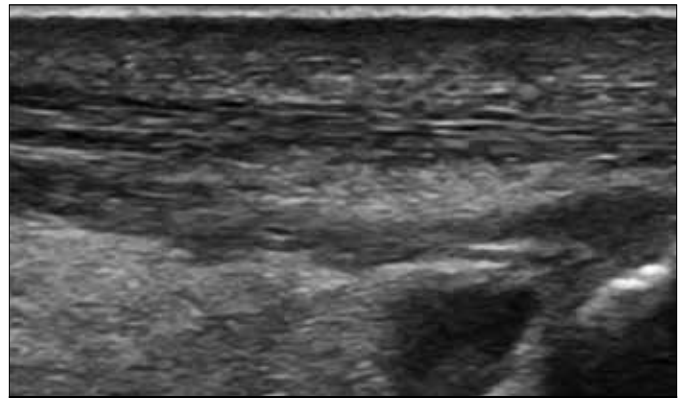


Figure 17. Case 3: Two months following single biocellular ARM treatment right achilles tendon. Previous tear improving with autograft still visible. New retrocalcaneal bursal fluid collection present.



Figure 18. Case 3: One year following biocellular ARM treatment. Note: normal appearance of Achilles tendon with resolution of tear and tendinosis.

Discussion

Tendinopathy and tears of the Achilles tendon are common in the population, and there currently exists no single best treatment option for those patients who suffer from Achilles tendinopathies, with or without partial tears. Tendons, when damaged, typically do not completely restore their full biological and biomechanical properties.^{7, 16, 59}

Use of the biologic of PRP has been used extensively for the treatment of tendinopathy in humans with anecdotal success and numerous reports that support its efficacy.^{1, 2, 14, 52} A recent article in the *JAMA* studied the use of PRP (only) versus saline control in a group of patients with Achilles tendon injury. Both groups improved beyond baseline equally, and the authors concluded that PRP injection was no more effective than a placebo effect and any benefit realized was due to the eccentric exercise program both groups followed post treatment.⁶⁰ No report of actual platelet concentrations achieved was made. This is significant considering that cellular proliferation and migration are markedly enhanced in a directly proportional fashion related to increasing platelet concentrations actually achieved.

This report had additional flaws. No fibrin gel or any extracellular matrix was employed with the PRP, and both the PRP treatment and the placebo group were treated with an eccentric exercise program. It is well established that eccentric exercise alone may lead to some improved outcomes.^{7, 52, 61} This particular study could have been improved if they either included a third control group isolating the effects of eccentric exercise only, or selected only subjects who had already undergone an eccentric exercise program and failed to improve. In addition, the diagnosis of tendinopathy was based upon subjective complaints and physical exam findings without use of diagnostic ultrasound evidence. Further, **without** documentation of actual individual patient platelet concentration (over measured baseline) counts mitigate against interpretation due to failure to quantify the actual PRP agent used in the injection process.

It is well established that not all PRP preparations are the same, and do not achieve equal platelet concentration.^{62, 63} This may significantly reduce the amount of growth factor and signal proteins delivered to the target site if not measured accurately. (See *Figure 1*.) There are

numerous platelet separation devices and techniques, from centrifuged blood tubes (single and double spin) to automated, closed mechanical separation, each of which make claims of concentration based primarily on extrapolated data. It is considered of great importance for the practitioner to be fully aware of the actual achieved concentrations in determining efficacy realized. In the *JAMA* report, only a single, small volume injection of PRP was administered, *without* mentioning any “needling” or “peppering” of the teno-osseous junction. Most reported PRP Prolotherapy (i.e., biologic only) uses require 4-6 repeated injections in order to achieve the optimal improvement in many tendon and ligament cases. Use of PRP only often falls short of full improvement or durability of result. In our experience, a series of 2-3 injections using the full ARM protocol every 8-12 weeks provides optimal results measured by anatomical findings and return to full activity without limitation of activity, elimination of discomfort, and with ultrasonic evidence of architectural improvement of the tendon structure.

Further studies are needed to address the efficacy of PRP alone versus ARM in treating tendonosis and tendinopathy.^{43, 64-66} We occasionally elect to try HD-PRP injections (only) in some acute tendon injuries, or to be tried when no distinct tear can be documented on diagnostic ultrasound. In general, the more chronic the problem, we believe there is an effective depletion of locally available cellular elements, which may limit the site’s ability to reach optimal healing. If there is any evidence of tear on MRI or diagnostic ultrasound, we recommend use of the ARM protocol as treatment therapy of choice.

As described, ARM is comprised of a combination of non-manipulated AD-tSVF (acquired by closed microcannula lipoaspiration), and either HD-PRP or BMAC. In select cases, the choice between use of HD-PRP and HD-BMAC is experienced-based decision on a protocol we have consistently employed for more than 7 years in the clinical setting. HD-PRP represents the most commonly used additive in our therapeutic protocol. Bone marrow may be selected as an additional additive in tendon tears greater than 3 cm in length, if treating concomitant cartilage injury/disease, or patients in whom 2 standard ARM treatments were incompletely successful. In the single case example in this reported series, bone marrow was combined with HD-PRP concentrates as an additional additive to the AD-tSVF.

A number of animal studies show great promise for use of isolated autologous MSCs mediated effects on tendon healing.^{2,35} In the human orthopedic literature the use of autologous MSC in tendon-ligament and bone healing are becoming more prevalent.^{67, 68} In addition, some practitioners include the use of either autologous or human recombinant thrombin as part of this protocol. Activation with thrombin stimulates immediate release of platelet granules, and contributes a fibrin gel product that aids the retention of the ARM grafts within the guided injection sites. Fibrin gel effectively reduces the potential of “drift” of fluids within existing fascial planes.⁵⁰ The use of autologous isolated MSC in peripheral joints and intervertebral discs has been reported to be safe and well tolerated in a study published in 2010.⁶⁹

Bone marrow stem cells have been extensively studied, with particular examination and evaluation of the marrow mesenchymal stem cell population (BM-MSC) for a variety of application in the human population. Recognition of the relative higher concentrations and ease of access to very similar cell populations having 90% overlap of the functional capabilities provided by adipose tissue has led to many more research and clinical studies. Recent research and clinical experiences have proven potential value of adipose-derived stem/stromal cells associated with provision of *MUCH* higher concentrations of mesenchymal and perivascular multipotent stromal cells. Further, laboratory findings relative the microenvironment, importance of secretory effects, and value of growth factor and signal protein supplementation have proven of value in applied clinical treatment of a variety of musculoskeletal applications, including tendon repair.

Adipose tissue complex has now been extensively characterized as having a very heterogeneous cellular population with important *native bioactive* scaffolding. Of major importance is higher availability (concentration) of adipose-derived mesenchymal stromal cells as compared to that found in bone marrow. Reports of such concentrations range from 1000-2500 times more MSCs concentrations native to adipose deposits compared to marrow.⁷⁰ With improving understanding of the pro-inflammatory and immune modulatory capabilities of the generic MSC populations, reports of striking improvements and value are accumulating rapidly in both research and translational applications.

Understanding the term “*Stromal Vascular Fraction (SVF)*” is important to recognize for the purposes of interpreting many studies and case reports. There are two terms often used interchangeably in the biomedical and clinical literature, and which, although similar, are significantly different. It is important for the reader to clearly establish which of the terms relative SVF is applied within the written study or report.

The *FIRST* term, known as “*Tissue*” SVF (tSVF), represents a non-digested, non-manipulated construct that contains the full heterogeneous cellular element, *plus* the native three-dimensional, bioactive extracellular matrix to which they closely relate. The *SECOND* term in common use in laboratory studies and certain clinical reports and is known as “*Cellular*” SVF. The cellular SVF (cSVF) product is developed following acquisition of adipose tissue complex (via excision or lipoaspiration), digestion of the tissue with collagenase/trypsin, agitation and incubation to separate the attached mononuclear non-designated cells from the extracellular matrix (ECM) and perivascular tissues. Following such manipulation, high g-force centrifugation is performed to isolate and concentrate those cells in pellet form. This effectively creates a cellular product devoid of its matrix that may be used in a variety of applications. In order to isolate specific subset cell populations, culture-expansion can be formed (with characterization) to separate the major cellular components. In clinical practice in the United States, regulatory issues preclude the use of this product, deeming it as “more than minimally manipulated” prohibits reinjection use in humans. It is hoped that these issues will be cleared, permitting the addition of more undifferentiated cellular elements to the treatment protocol.⁴⁰

In cosmetic-plastic surgery, addition of the cSVF components *BACK INTO* non-manipulated autologous lipoaspirants has been termed “cell-assisted” lipoplasty. Reports of enhanced lipogenic volume retention in structural fat grafting procedures suggest value in provision of additional cSVF cells to the graft.⁷¹ It should be understood that this does not represent **any** increase over native baselines within adipose tissue, but rather restoration of a number of cells lost during the process of lipoaspiration of graft (versus en bloc excision samples). Lipoaspiration has been shown to return slightly more than 53% of the total available cSVF concentrations existing within native fat microenvironments. It is considered likely

that, when regulatory issues are addressed, use of this “cell-assisted” protocol will prove of value by providing more cells and perhaps further enhance responsiveness to HD-PRP additive concentrates as used in the ARM protocol.

It is important to reiterate that, the biocellular ARM application described within this document uses only non-manipulated, microcannula-syringe lipoaspirated, autologous adipose tSVF products only, and we have utilized adipose-derived tissue SVF (AD-tSVF) in our therapeutic protocols.

Adipose deposits are typically readily available for harvest and offer very similar types of undifferentiated mesenchymal and perivascular cell populations as marrow. AD-tSVF offers higher availability, less donor site morbidity, ease of harvest, less cost, and is well tolerated when placed into injured or damaged tissues compared to use of marrow. The enhanced value available in the planned combination of AD-tSVF plus high-density platelet-rich plasma (HD-PRP) concentrates has rapidly gained acceptance.^{59, 72, 73} We believe that using **only** HD-PRP (defined as being >4 times actual measured patient baselines) deserves important emphasis. It is considered essential to interpret or analyze published articles on clinical experiences using any PRP concentrates to determine the platelet density utilized. There is a direct correlation between achieved platelet concentration and the quantitative levels of additional growth factor-signal proteins delivered. This has been shown to materially and substantially impact the microenvironmental effects clinically achieved.

At this time, reports of several controlled clinical trials are beginning to be published. Several thousand successful guided, percutaneous placement of the Autologous Regenerative Matrix (ARM), provides a remarkable safety and efficacy and experiential record that should not be ignored offhand. Many papers like this one report on the values achieved in the musculoskeletal applications.

We present representative case reports effectively utilizing this technique, specifically in the Achilles tendon area. Although this case series focuses on the difficult to treat injury group, the Achilles tendon, use of such protocols have led us to extensively utilize the same protocol on other tendons, ligaments, muscle injuries, cartilage-bone, and joint issues (including osteoarthritis).

As in all small case series reports, limitation of interpretation is present. The cases here presented are highly representative of many experiences using AD-tSVF with autologous additive in the treatment protocol. Technically speaking, it is believed that the important combination lies in the *integration* of the AD-tSVF (with its important structural, cellular and paracrine potentials) with a concentrated source of important platelet-derived growth factors and signal proteins. It remains the author’s opinion that the use of AD-tSVF with *EITHER* HD-PRP concentrates (or BMAC in select cases) falls within the term of Autologous Regenerative Matrix (ARM) represents an effective therapeutic modality. Taking advantage of important AD-tSVF elements plus select additives provides key structural scaffolding, bioactive matrix, heterogeneous cellular populations, and potent autocrine-paracrine influences, is believed to offer higher efficacy than can be attained by any individual component parts alone.

Thoughts exist that the combination of AD-tSVF and BMAC (only) in complex tendon and musculoskeletal injuries also offer a potential therapeutic modality.⁴⁴ One of the limiting factors for selection of BMAC as an additive to the AD-tSVF is that it requires disposable durable “kits” which are very costly. Also, the harvesting procedure for BMAC is more invasive and often less favorably perceived by patients.

Examination the heterogeneous components within AD-tSVF have been extensively studied and reported relative the total number of mononuclear cell counts, existence of several important sub-populations, high viability, extensive cell characterization, and culture expansion potentials *In Vitro*. It is well documented that there are approximately one million of such cells per cc of microcannula harvested lipoaspirant.⁷⁴ The optimal number of stem/stromal cells for transplantation or levels specific chemical signal elements have yet to be determined, and is the current focus of multiple research efforts.

Some reports advocate isolation, concentration and culture-expansion of autologous MSC’s prior to guided re-implantation have been recommended as potentially advantageous. These isolation and expansion methods currently are outside the current U.S. Food and Drug Administration’s Regulatory Guidelines and are defined as more than minimally manipulated (MMM) and remain controversial.

Favoring use of raw ATC and additives versus the isolation-concentration and/or use of expanded specific cell populations is gaining importance. In a recent contribution in *Translational Medicine* (Sept 2012) by Herbert et al, complex analyses provide very clear evidence of the *greater potency potential* when the AD-tSVF is introduced intact, permitting the entire group of components to contribute according to the target site needs and abilities. This extensive proteome analysis demonstrated the statistical advantages when adipocytes and their investing SVF are permitted to interact by cell-to-cell, autocrine and paracrine signaling.⁷⁵ We believe our extensive successful clinical experiences with the ARM protocol support these findings and may explain the excellent safety and efficacy observed in use of the presented therapeutic modality. Limitations of translation from “bench-to-beside” results in using *In Vitro* findings which may not mimic the native, 3-dimensional, biological microenvironmental issues of *In Vivo* niches. At this point in time, extensive chemical manipulations to isolate a single individual cell types, or select specific growth factors and chemical factors to add, is not practical. Current clinical practice does not yet have the knowledge to select *THE KEY* specific cellular or chemical factors, or the optimal concentrations most needed at an injury or degenerative site. Completion of several thousand cases within the cosmetic-plastic and MSK application areas supports the use of the logic of combination in ARM therapy. Ease of delivery in point-of-care centers, within the same day, using same patient autologous criteria, within the same surgical procedure it is currently believed to fall within the Practice of Medicine category without violating existing regulatory issues.

Conclusion

We introduce a biocellular modality representing the use of AD-tSVF plus HD- PRP concentrates and/or BMAC. We describe the Autologous Regenerative Matrix (ARM) as a protocol to provide safe and efficacious therapeutic modality for use in a variety of musculoskeletal applications. Use of ultrasonically guided, percutaneous treatment delivery to target specific injured or damaged tissues has produced an excellent, reproducible, track record relative patient responses in repair and regenerative cases. The rate of success in restoration of tendon and function rivals that of any other treatment

modality at this time (including open surgical repair), and significantly reduces time required for rehabilitation, pain reduction, and return to full function.

Achilles tendinopathy and tears are recognized to represent a problematic treatment group in order to achieve complete healing and restoration of function. In the case of tendon injury or degeneration, return to completely normal function and load capabilities following surgical intervention or guided therapy is not uniformly possible. In fact, re-injury producing subsequent pain, poor function, and even subsequent rupture has been reported many times. Recently, recognition of use of AD-stem/stromal tissue SVF (AD-tSVF) and biologic additives to provide a variety of cellular-chemical elements with important native bioactive scaffolding, show great promise in promoting healing potential in many orthopedic applications (including tendon injury and related disorders). Use of AD-tSVF with **either** HD-PRP concentrates or BMAC additives are proving safe and efficacious in a variety of aesthetic, reconstructive, and musculoskeletal applications. More extensive research needs to be performed, but use of non-manipulated adipose tissue complex with highly concentrated growth factors-signal proteins as part of an ARM presents an attractive option for treating tendon injuries such as Achilles tendinopathy with moderately severe and partial tears. ■

REFERENCES

1. Wilkins R, Bisson L. Operative versus nonoperative management of acute achilles tendon rupture. *Am J Sports Med.* 2002;30:783-790.
2. Gulotta L, Chaudhury S, Wiznia D. Stem cells for augmenting tendon repair. *Stem Cells International.* 2012;1-7.
3. Popovic N, Lemaire R. Diagnosis and treatment of acute ruptures of the achilles tendon. Current concepts review. *Acta Ortho Bel.* 1999;65:464-469.
4. Caplan AI. The mesengenic process. *Clinics in Plastic Surgery.* 1994;21(3):429-435.
5. Mauffulli N, Wong J, Almekinders LC. Types and epidemiology of tendinopathy. *Clin Sports Med.* 2003;22(4):675-692.
6. Alfredson H, Lorentzon R. Chronic Achilles tendinosis: recommendations for treatment and prevention. *Sports Med.* 2000;29:135.
7. Kingma J, de Knikker R, Wittink H, Takken T. Eccentric overload training in patients with chronic achilles tendinopathy: a systematic review. *Br J Sports Med.* 2007;41:e3.
8. Khan RJ, Fick D, Keogh A, et al. Treatment of acute achilles tendon ruptures. A meta-analysis of randomized, controlled trials. *J Bone Joint Surg Am.* Oct 2005;87(10):2202-10.

9. Schepsis AA, Jones H, Haas AL. Achilles tendon disorders in athletes. *Am J Sports Med.* Mar-Apr 2002;30(2):287-305.
10. Keene JS. Tendon injuries of the foot and ankle. In: DeLee JC, Drez D, eds. *Orthopaedic Sports Medicine.* Philadelphia, Pa: WB Saunders. 1994:1768-1805.
11. Leppilahti J, Ovava S. Total achilles tendon rupture. A review. *Sports Med.* 1998;25:79.
12. Kujala UM, Sarna S, Kaprio J. Cumulative incidence of achilles tendon rupture and tendinopathy in male former elite athletes. *Clin J Sport Med.* 2005;15:133.
13. Maffulli N, Longo UG, Maffulli GD, Khanna A, Denaro V. Achilles tendon ruptures in elite athletes. *Foot Ankle Int.* Jan 2011;32(1):9-15.
14. Alfredson H. Chronic midportion achilles tendinopathy: an update on research and treatment. *Clinics in Sports Medicine.* 2003;22(4):727-41
15. Mauffuli N, Sharma P, Luscombe K. Achilles tendinopathy, aetiology and management. *J R Soc Med.* 2004;97(10):472-476.
16. Rompe J, Furia J, Maffulli N. Eccentric loading compared with shock wave treatment for chronic insertional achilles tendinopathy. a randomized, controlled trial. *J Bone Joint Surg Am.* 2008;90:52-61.
17. De Jonge S, de Vos R, Weir A, van Schie H, Bierma-Zeinstra S, Verhaar J, et al. One year follow-up of platelet rich plasma treatment in chronic achilles tendonopathy: a double blind randomized placebo-controlled trial. *Am J Sports Med.* 2011;39(8):1623-1629.
18. Monto R. Platelet rich plasma treatment for chronic achilles tendinopathy. *Foot Ankle Int.* 2012;33(5):379-385.
19. Pufe T, Petersen W, Tillmann B, Mentlein R. The angiogenic peptide vascular endothelial growth factor is expressed in foetal and ruptured tendons. *Virchows Archive.* Oct. 2001;439(4):579-585.
20. Soma CA, Mandelbaum BR. Repair of acute achille tendon ruptures. *Orthop. Clin. North Am.* 1995;26:239-247.
21. Cetti R, Christensen S, Ejsted R, Jensen N, Jorgensen U. Operative versus non-operative treatment of achilles tendon rupture. *Am J of Sports Med.* 1993;21:791-799.
22. Metzi JA, Ahmad CS, Levine WM. The ruptured achilles tendon: operative and non-operative treatment options. *Curr Rev Musculoskelet Med.* 2008;1(2):161-164.
23. Chalmers J. Review article: Treatment of achilles tendon ruptures. *J of Orth Surgery.* 2000;8(1):97-99.
24. Khan RJK, Carey Smith R. Surgical interventions for treating acute achilles tendon ruptures. *The Cochrane Library Summaries,* Published Online: September 8, 2010.
25. Uysel A, Mizuno H. Tendon regeneration and repair with adipose derived stem cells. *Current Stem Cell Res & Therapy.* 2010;5:161-167.
26. Manning C, Schwartz A, Liu W, Xie J, Havlioglu N, Sakiyama-Elbert S, et al. Controlled delivery of mesenchymal stem cells and growth factors using nanofiber scaffold for tendon repair. *Acta Biomater.* 2013;2:epub.
27. de Mos M, van der Windt A, Jahr H, van Schie H, Weinans H, Verhaar J, et al. Can platelet-rich plasma enhance tendon repair? a cell culture study. *Am. J. Sports Med.* 2008;36:1171-1178.
28. Anitua E, Andia I, Sanchez M, Azofra J, del Mar Zaldueño M, de la Fuente M, et al. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. *J Orthoped Res.* 2005;23(2):281-286.
29. Molloy T, Wang Y, Murrell. The roles of growth factors in tendon and ligament healing. *Sports Med.* 2003;33(5):381-394.
30. Gulotta L, Kovacevic D, Montgomery S, Ehteshami J, Packer J, Rodeo S. Stem cells genetically modified with the developmental gene MT1-MMP improve regeneration of the supraspinatus tendon-to-bone insertion site. *Am J Sports Med.* 2010;38(7):1429-1437.
31. Alderman D, Alexander R. Advances in regenerative medicine: high-density platelet-rich plasma and stem cell prolotherapy for musculoskeletal pain. *Prac Pain Management.* 2011;10:49-90.
32. Eagan M, Zuk P, Bluth B, Brinkmann E, McAllister D. The suitability of human adipose-derived stem cells for engineering of ligament tissue. *J. Tissue Eng & Regen Med.* 2011.
33. James R, Kesturu G, Balian G, Chhabra A. Tendon: biology, biomechanics, repair, growth factors, and evolving treatment options. *J. Hand.Surg Am.* 2008;33(1):102-112.
34. Zhang F, Liu H, Stile F, Lei M, Pang Y, Oswald T, et al. Effect of vascular endothelial growth factor on rat achilles tendon healing. *Plast Recon Surg.* 2003;112(6):1613-1619.
35. Nixon A, Dahlgren L, Haupt J, Yeager A, Ward D. Effect of adipose-derived nucleated cell fractions on tendon repair in horses with collagenase-induced tendinitis. *Am J. Vet Res.* 2008;69:928-937.
36. Alderman D, Alexander RW. Stem cell prolotherapy in regenerative medicine. *J Prolotherapy.* 2012; 3(3): 689-706.
37. Choi J, Minn K, Chang H. The efficacy and safety of platelet-rich plasma and adipose-derived stem cells: an update. *Arch Plast Surg.* 2012;39(6):585-592.
38. Alsousou J, Ali A, Willett K. The role of platelet-rich plasma in tissue regeneration. *Platelets.* 2013;24(3):173182.
39. MacLean S, Khan W, Malik A, Snow M, Anand S. Tendon regeneration and repair with stem cells. *Stem Cells International.* vol. 2012;Article ID 316281.1-6.
40. Alexander RW. Understanding Adipose-derived stromal vascular fraction (AD-SVF) cell biology and use on the basis of cellular, chemical, structural and paracrine component: a concise review. *Journal of Prolotherapy.* 2012;4:e855-e869.
41. Alexander RW. Autologous fat grafts as mesenchymal stromal-stem cell source for use in prolotherapy: a simple technique to acquire lipoaspirants. *J Prolotherapy.* 2012.
42. Alexander RW. Use of platelet-rich plasma to enhance effectiveness of autologous fat grafting. In *Autologous Fat Grafting, 1st Ed.* Springer-Verlag Berlin Heidelberg, ed. Shiffman M, 2010.

43. *In Press*: Tate K, Crane C. Platelet rich plasma and biocellular grafts. In: Lana J, Santana M, Luzo A, Belangero W, editors. *Regenerative Medicine - Platelet Rich Plasma: The State of the Art*. Springer; 2013.
44. Strioga M, Viswanathan S, Darinskas A, Slaby O, Michalek J. Same or not the same? comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. *Stem Cells Dev*. 2012;21(14):2724-2752.
45. Alexander R. Liposculpture in the superficial plane: closed syringe system for improvements in fat removal and free fat transfer. *Am J Cosm Surg*. 1994;11(2):127-134.
46. Barnett J, Pomeroy G. Use of platelet- rich plasma and bone marrow derived mesenchymal stem cells in foot and ankle surgery. *Techniques in Foot and Ankle Surgery*. 2007;6(2):89-94.
47. Suga H, Eto H, Aoi N, Kato H, Araki J, Doi K, et al. Adipose tissue remodeling under ischemia: death of adipocytes and activation of stem/stromal progenitor cells. *Plast Reconstr Surg*. 2010;126(6):1911-1923.
48. Pufe T, Petersen W, Kutz B, Tsokos M, Tillmann B, Mentlein R. Mechanical factors influence the expression of endostatin – an inhibitor of angiogenesis in tendons. *J. Orthop Res*. 2003;21(4):610-616.
49. Pufe T, Petersen W, Mentlein R, Tillmann B. The role of vasculature and angiogenesis for the pathogenesis of degenerative tendon disease. *Scand J. Med Sci Sports*. 2005;15(4):211-222.
50. Petersen W, Pufe T, Pfrommer S, Tillmann B. Overload damage to achilles tendon: the importance of vascularization and angiogenesis. *Orthopade*. 2005;34(6):533-542.
51. Gimble J, Katz A, Bunnell B. Adipose-derived stem cells for regenerative medicine. *Circ Res*. 2007;100:1249-1260.
52. Tapp H, Hanley EN Jr, Patt JC, Gruber HE. Adipose-derived stem cells: characterization and current application in orthopaedic tissue repair. *Exp Biol Med*. 2009;234:1-9.
53. Guilak F, Estes B, Diekman B, Moutos F, Gimble J. Multipotent adult stem cells from adipose tissue for musculoskeletal tissue engineering. *Clin Orthop Relat Res*. 2010;9:2530-2540.
54. Alexander RW. Autologous fat grafts as mesenchymal stromal stem cell source for use in prolotherapy: a simple technique to acquire lipoaspirants. *J of Prolotherapy*. 2011;3(3):680-688.
55. Albano J, Alexander RW. Autologous fat grafting as a mesenchymal stem cell source and living bioscaffold in a patellar tendon tear. *Clin J. of Sports Med*. 2011;21(4):359-361.
56. Gentile P, Orlandi A, Scioli M, Di Pasquah C, Bocchini I, Cervelli V. Concise review: adipose-derived stromal vascular fraction cells and platelet-rich plasma: basic and clinical implications for tissue engineering therapies in regenerative surgery. *Stem Cells Translat. Med*. 2012;1(3):230-236.
57. Gamradt S, Rodeo S, Warren R. Platelet-rich plasma in rotator cuff repair. *Tech in Orthoped*. 2007;22(1):26-33.
58. Savitskaya Y, Izaguirre A, Sierra L, Perez F, Cruz F, Villalobos E, et al. Effect of angiogenesis-related cytokines on rotator cuff disease: the search for sensitive biomarkers of early tendon degeneration. *Clin Med Insights: Arthritis & Musculoskel Disorders*. 2011;4:k43-53.
59. Alfredson H, Cook J. A treatment algorithm for managing achilles tendinopathy: new treatment options. *Brit J Sports Med*. 2007;41(4):211-216.
60. DeVos R, Weir A, van Schie H, Bierma-Acintra S, Verhaar J, Weinans H, et al. Platelet rich plasma injection for chronic achilles tendinopathy. *JAMA*. 2010;303(2):144-149.
61. Alfredson H, Pietila T, Jonsson P, Lorentzon R. Heavy-load eccentric calf muscle training for the treatment of chronic achilles tendinosis. *Am J Sports Med*. 1998;26:360-366.
62. Mazzocca A, McMarthy M, Chowanec D, Cote M, Romeo A, Bradley J, et al. Platelet-rich plasma differs according to preparation method and human variability. *Am. J. Bone Joint Surg*. 2012;94(4):308-316.
63. Alsousou J, Thompson M, Hulley P, Noble A, Willett K. The biology of platelet-rich plasma and its application in trauma orthopedic surgery. *J. Bone Joint Surg (Br)*. 2009;91(8):987-996.
64. Sanchez M, Anitua E, Azofra J, Andia I, Padilla S, Mujika I. Comparison of surgically repaired achilles tendon tears using platelet-rich fibrin matrices. *Am J Sports Med*. 2007;35(2):245-251.
65. Sanchez M, Anitua E, Andia I. Application of autologous growth factors on skeletal muscle healing. *2nd World Congress on Regenerative Medicine. Podium Presentation*. May 2005.
66. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet rich plasma. *AM J Sports Med*. 2006;10(10):1-5.
67. Uysal A, Mizuno H. Tendon regeneration and repair with adipose derived stem cells. *Current Stem Cell Research Therapy*. 2010;5:161-167.
68. Rush SM. Trinity evolution: mesenchymal stem cell allografting in foot and ankle surgery. *Foot & Ankle Specialist*. 2010;3(3):140-143.
69. Goldschlager T. Potential applications for using stem cells in spine surgery. *Current Stem Cell Research and Therapy*. 2010;5(4):345-355.
70. Alexander RW. Fat transfer with platelet-rich plasma for breast augmentation. In *Autologous Fat Grafting, 1st Ed*. Springer-Verlag Berlin Heidelberg, ed. Shiffman M, 2010.
71. Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T. Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. *Aesth Plast Surg*. 2008;32(1):48-55.
72. Centeno C, Schultz J, Cheever M, Robinson B, Freeman M, Marasco W. Safety and complications reporting on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. *Current Stem Cell Research and Therapy*. 2010;5(1):81-93.
73. Kryger GS, Chong A, Costa M, Pham H, Bates S, Chang J. A comparison of tenocytes and mesenchymal stem cells for use in flexor tendon tissue engineering. *J Hand Surg Am*. 2007;32(5):597-605.
74. Alexander R, Mandle R. Unpublished data Harvard Biosciences Research Laboratory, Cambridge, MA.
75. Blaber S, Webster R, Cameron J, Breen E, Kuah D, Vesey G, Herbert B. *J of Translational Med*. 2012;10:172.