



## Original Research

# Randomized, Placebo-Controlled Analysis of the Knee Synovial Environment Following Platelet-Rich Plasma Treatment for Knee Osteoarthritis

Jason D. Tucker, MD , Lance L. Goetz, MD, Michael B. Duncan, PhD, Jared B. Gilman, MD, Lynne W. Elmore, PhD, Scott A. Sell, PhD, Michael J. McClure, PhD, Peter V. Quagliano, MD, Caroline C. Martin, MD

---

## Abstract

**Background:** Platelet-rich-plasma (PRP) is used to treat knee osteoarthritis; however, mechanistic evidence of PRP effectiveness for pain relief is limited.

**Objective:** To assess molecular biomarkers and mesenchymal stem cells (MSCs) in synovial fluid during PRP treatment of the osteoarthritic knee joint.

**Design:** Single blinded, randomized, placebo controlled pilot study.

**Setting:** Veterans Affairs Medical Center.

**Participants:** Seventeen participants with mild to moderate knee osteoarthritis were randomized in a 2:1 placebo-controlled ratio, receiving PRP or saline (placebo) intra-articular injection into the knee joint.

**Methods:** Knee synovial fluid was analyzed before the respective injections and again 10 days following injection. Participants were followed up to 12 months completing visual analog scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaires at intervals over that period.

**Main Outcome Measures:** The effects of PRP on synovial protein and MSC gene expression levels were measured by multiplex enzyme-linked immunosorbent assay and quantitative polymerase chain reaction.

**Results:** Novel biomarkers including levels of interleukin (IL)-5, IL-6, IL-10, and tumor necrosis factor- $\alpha$  were measured in synovial fluid 10 days after PRP treatment. Altered gene expression profiles in MSCs from patients treated with PRP were observed for matrix metalloproteinases and inflammatory markers (IL-6, IL-8, CCL2, TNF- $\alpha$ ). A2M protease was significantly increased following PRP treatment ( $P = .005$ ). WOMAC scores declined for up to 3 months from baseline levels and remained low at 6 and 12 months in the PRP group. In contrast, WOMAC scores for patients receiving the saline injection were relatively unchanged for up to 12 months.

**Conclusions:** We report significant changes for the biomarker A2M ( $P = .005$ ) as well as differences in expression of cellular markers and postulate that PRP modulates the local knee synovial environment by altering the inflammatory milieu, matrix degradation, and angiogenic growth factors. The PRP treatment group had less pain and stiffness and improved function scores.

---

## Introduction

Knee osteoarthritis (KOA) is an inflammatory and degenerative disease that causes pain, stiffness, and functional impairment. It is estimated that ~20 million Americans have KOA-related pain.<sup>1</sup> The incidence of KOA is rising because of both an increasing average age and a growing obesity epidemic.<sup>1-3</sup> Although there is evidence that the

pathology of KOA is related to breakdown of joint tissues owing to mechanical stress and inflammatory factors,<sup>4,5</sup> there is no definitive pathophysiologic mechanism describing how healthy cartilage deteriorates to cause pain. There are three main areas where malfunction occurs: cartilage, subchondral bone, and synovial tissue.<sup>6-8</sup> Preclinical studies suggest that cartilage repair is feasible, but the exact mechanisms involved remain unclear.<sup>9,10</sup>

Although it was postulated that platelet-rich plasma (PRP) can promote the healing of damaged tissue, only limited evidence indicates that restorative factors are released by platelets.<sup>11</sup> There is no clinical evidence to date that PRP alone can stimulate cartilage regrowth or healing.

Putative biomarkers play destructive, protective, and restorative roles in joint homeostasis and tissue breakdown during KOA. "Destructive" markers are thought to include matrix metalloproteases (MMPs), cartilage oligomeric matrix protein (COMP), proinflammatory interleukins (eg, IL-1 $\beta$ ), tumor necrosis factor (TNF- $\alpha$ ), lipids, and nitric oxide.<sup>12,13</sup> "Protective" markers include tissue inhibitors of matrix metalloproteases (TIMPs), anabolic cytokines (eg, insulin-like growth factor-1 [IGF-1], fibroblast growth factor [FGF], bone morphogenic proteins [BMPs]), and platelet-derived growth factor [PDGF]).<sup>14-18</sup> These destructive/protective cytokines and growth factors regulate stem cell activation and differentiation in soft tissues. Whether they also play a role in regulating synovial mesenchymal stem cells (MSCs) remains unclear. MSCs are involved in soft tissue restoration and homeostasis, possessing the ability to self-renew and produce progeny that differentiate.<sup>19,20</sup> MSCs secrete factors that regulate KOA-related pathways<sup>19,20</sup> and the immune response.<sup>21</sup> In vitro studies indicate that MSCs are responsive to PRP, demonstrating enhanced proliferation, secretion of modulatory factors, and differentiation potential.<sup>22-25</sup> These results suggest that PRP may stimulate a restorative response through the activation of MSCs within synovial fluid (SF).

Conservative treatment options (eg, weight loss, exercise, orthotics, and systemic/local pharmacological interventions) aim to postpone symptomatic joint degeneration and progression to joint replacement. Joint replacement comes with risks of patient dissatisfaction and serious postoperative complications, including but not limited to infection, prosthesis failure, stroke, myocardial infarction, and increased risk of deep venous thrombosis.<sup>26</sup> Emerging treatment options focus on reversing the degenerative cascade while avoiding the need for invasive surgical interventions. PRP is becoming a more common option and some systematic reviews report short-term clinical benefit for up to 6-12 months in Kellgren-Lawrence (KL) II-III KOA.<sup>27-29</sup> However, mechanistic evidence of how PRP achieves pain relief is limited. Additionally, there are few mechanistic clinical studies on the effects of PRP on the synovial joint space. This lack of insight limits the development of PRP formulations specific for KOA.

The aim of this study was to determine the feasibility of assessing SF biomarkers across a participant pool, in order to understand the molecular and cellular responses that PRP mediates in the osteoarthritic joint. We hypothesized that biochemical changes in the joint space occur within SF following PRP treatment, favoring pain relief and suppressing classically activated markers of KOA. Synovial biomarkers of inflammation, tissue

remodeling, and MSC characteristics were measured in conjunction with clinical and imaging outcomes to identify potential mechanisms for improved clinical outcomes associated with a PRP-based treatment strategy.

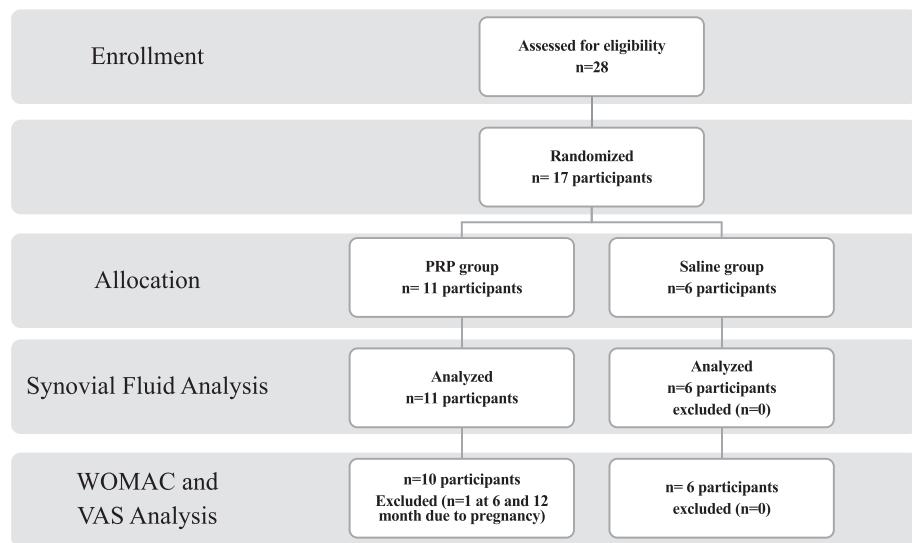
## Methods

### *Trial Design and Participants*

This was a randomized, controlled, single-blinded, pilot study with two groups: a PRP intervention and a saline control. Participants were solicited from the population of veterans who were being seen in outpatient clinics (orthopedics, physical medicine and rehabilitation, primary care) with a diagnosis of knee osteoarthritis. Participants had to be 40 years of age or older with diagnosis of KL II-III KOA. Specific inclusion/exclusion criteria are provided in Table S1. A total of 17 participants who met inclusion criteria were enrolled and randomly assigned in a 2:1 ratio using a random number generator. Eleven participants were randomized to the PRP intervention group and six to the saline control group. Participants were blinded to their grouping for treatment. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research. The institutional review board at the McGuire VA Medical Center approved the protocol, and all participants provided written informed consent.

### *Blood Draws and Manufacture of PRP*

Blood draws took place using standard precautions. At the study visit, all participants had blood drawn and processed for PRP manufacture and analysis; however, only intervention participants received intra-articular PRP injections. The PRP was manufactured according to the device guidelines (as per the 510(k)-approved insert). Participants (N = 17) had ~240 mL of peripheral blood drawn into syringes (using an 18-20-gauge needle) pretreated with anticoagulant citrate dextrose (ACD-A) solution (standard anticoagulant-treated tube from the Autologous Platelet-Integrated Concentrate (APIC)-PRP kit). Approximately 8 mL of PRP were produced for each patient. For the intervention group, the PRP that was derived from the venipuncture was centrifuged in a Cynetics APIC PRP System. The blood draw and processing to PRP for participants occurred at the same visit to minimize wait time and avoid repeat knee joint needle stick (ie, for injection with PRP study product into the knee joint), with the goal of reducing inconvenience, discomfort and infection risk. All participants had ~3 mL of PRP divided for further testing: 1 mL for complete blood count (McGuire VA Medical Center Pathology Lab), 1 mL for biomarkers /cytokines, and 1 mL for cellular/biochemical analysis. Remaining fluid was stored (-80°C) for additional analyses. Individuals in the control group had



**Figure 1.** Study enrollment. Chart showing enrollment of study participants, allocation of study groups, biochemical, and clinical analysis. PRP = platelet-rich plasma; VAS = visual analog scale; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index.

laboratory analyses of PRP but received a saline injection into the knee joint. All laboratory results were blinded.

### SF Aspiration and PRP Injection

Under aseptic technique with a surgical drape/curtain in place to block the study participant's view of the knee, SF samples were obtained from all participants by an ultrasound-guided standard superolateral approach.<sup>30</sup> To reduce a volume-based confounding factor, all fluid was aspirated until there was no further discernible fluid visible on imaging.<sup>31</sup> Each intervention participant had 5 mL PRP injected and the control participants had 5 mL saline injected. To reduce participant discomfort, eutectic mixture local anesthetics cream was applied to the skin prior to insertion of an 18-gauge needle into the knee joint space. Participants returned for SF aspiration at 10 days ( $\pm 2$  days), which was completed under aseptic technique and with ultrasound guidance. In one case, very little effusion was present at this time point. Saline was injected into the knee joint followed by complete aspiration to allow for SF analysis. This method has worked in prior studies to calculate SF quantitatively for volume based on urea concentration but has not been used for qualitative data.<sup>32</sup> Participants were instructed not to use nonsteroidal anti-inflammatory drugs, ice, or heat unless absolutely necessary for 48 hours before baseline appointment and through follow-up #1 (Day 10). They were instructed to rest for 24 hours and provided tramadol for postinjection pain relief and standard doses of acetaminophen were permitted. Participants were provided information on pain medication restrictions and an exercise/activity journal.

### Outcomes Measure

Participants completed a 100-point visual analog scale (VAS) and 100-point Western Ontario and McMaster (WOMAC) arthritis questionnaire at baseline, Day 10 follow-up and at the 3, 6, and 12-month time period from baseline (initial PRP injection). VAS scores were assessed via participants placing a mark on a standard design 10-cm line. Knee radiographs were taken at baseline and at the 6-month follow-up.

### SF Analysis

Specimens were coded and de-identified before sending to laboratories for analyses. SF aspirate was collected, and complete blood cell counts were analyzed for patients randomly assigned in control (saline) and PRP treatment groups at the time of PRP treatment (NT, no treatment).

**Table 1**  
Baseline characteristics of patients included in the study

	Saline	PRP	P Value
Patients (n)	6	11	
Gender, female, n (%)	4 (66.7%)	3 (27.3%)	.11
Age, mean	57.2 $\pm$ 3.9	57.5 $\pm$ 1.8	.94
Body mass index, mean	29.1 $\pm$ 2.1	30.9 $\pm$ 1.5	.51
Pain	35.6 $\pm$ 8.1	47.7 $\pm$ 8.9	.46
Stiffness	52.5 $\pm$ 9.9	55.9 $\pm$ 9.8	.92
Physical function	43.4 $\pm$ 8.4	48.9 $\pm$ 7.7	.88
VAS	37.9 $\pm$ 9.2	48.7 $\pm$ 9.8	.69

Values are the average and include  $\pm$  SEM. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores for pain, stiffness, and physical function as well as visual analog scale (VAS) are scored on a 100-point scale. For the WOMAC and VAS higher scores for pain, stiffness, and physical function are associated with increased pain, stiffness, and difficulty, respectively.

**Table 2**

Baseline serum and PRP platelet and white blood cell counts

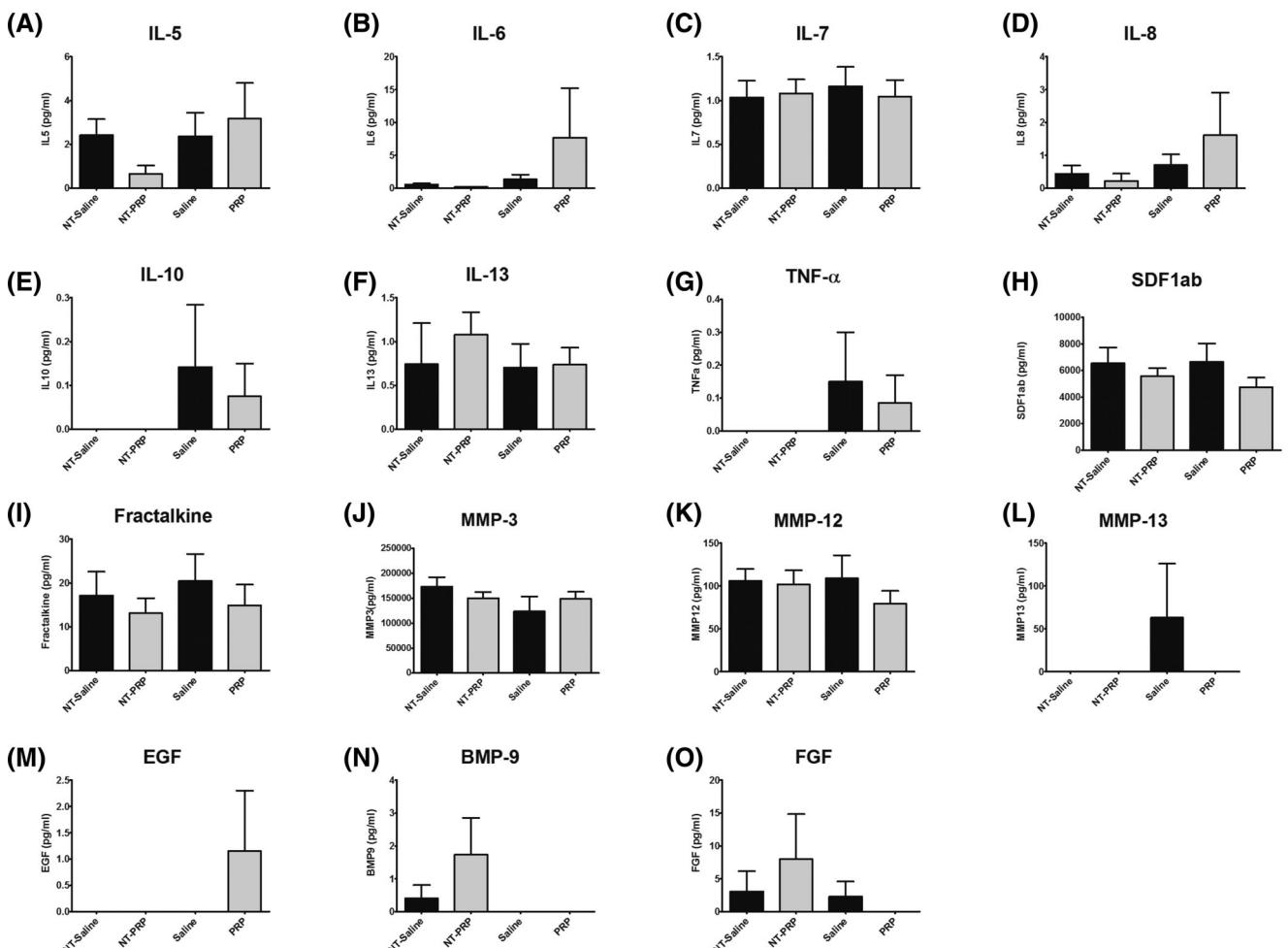
	Whole Blood	PRP	Fold Change	% Increase
White blood cell	6.78 ± 2.47	2.61 ± 0.98	0.43x ± 0.23	
Red blood cell	4.41 ± 0.78	ND		
Hemoglobin	13.49 ± 2.26	ND		
Hematocrit	39.58 ± 5.96	0.40 ± 0.13		
Platelet count	264.91 ± 62.79	703.73 ± 308.32	2.59x ± 0.68	158 ± 67%
Platelet load		3.52 ± 1.54		

Values are the average and include ±SD. Red blood cell and hemoglobin levels for platelet-rich plasma were below the detection limit and therefore were not determined (ND). White blood cells (million cells/mL), red blood cells (billion cells/mL), hemoglobin (grams/dL), hematocrit (%), Platelet count (million/mL), Platelet load (billion counts).

PRP = platelet-rich plasma.

and again 10 days (±2 days) post PRP or saline. (See Figure S1) using a Sysmex XN 9000 CBC analyzer [Sysmex Corp., Kobe, Japan].) Residual SF was centrifuged and divided into supernatant and cell portions. The cellular portion was used to isolate MSCs. The supernatant was separated into aliquots for freezing (-80°C). Protein

biomarker samples were analyzed via Luminex and multiplexing enzyme-linked immunosorbent assay (ELISA). Samples were de-identified and sent out according to institutional biohazard sample handling procedures. Laboratories in the study are Clinical Laboratory Improvement Amendments certified.



**Figure 2. Analysis of immunomodulatory and tissue remodeling biomarkers following PRP treatment.** Cytokine levels were assessed by multiplex ELISA in synovial fluid aspirate before and 10 days following PRP or saline treatment. No treatment (NT) indicates baseline levels for the cytokine prior to the patient receiving saline or PRP treatment. One way analysis of variance with Tukey multiple comparison post-hoc test ( $P$  values  $< .05$  were considered significant). BMP = bone morphogenic protein; EGF = epidermal growth factor; FGF = fibroblast growth factor; IL = interleukin; MMP = matrix metalloproteases; PRP = platelet-rich-plasma; SDF1 = stromal cell-derived factor 1; TNF- $\alpha$  = tumor necrosis factor alpha.

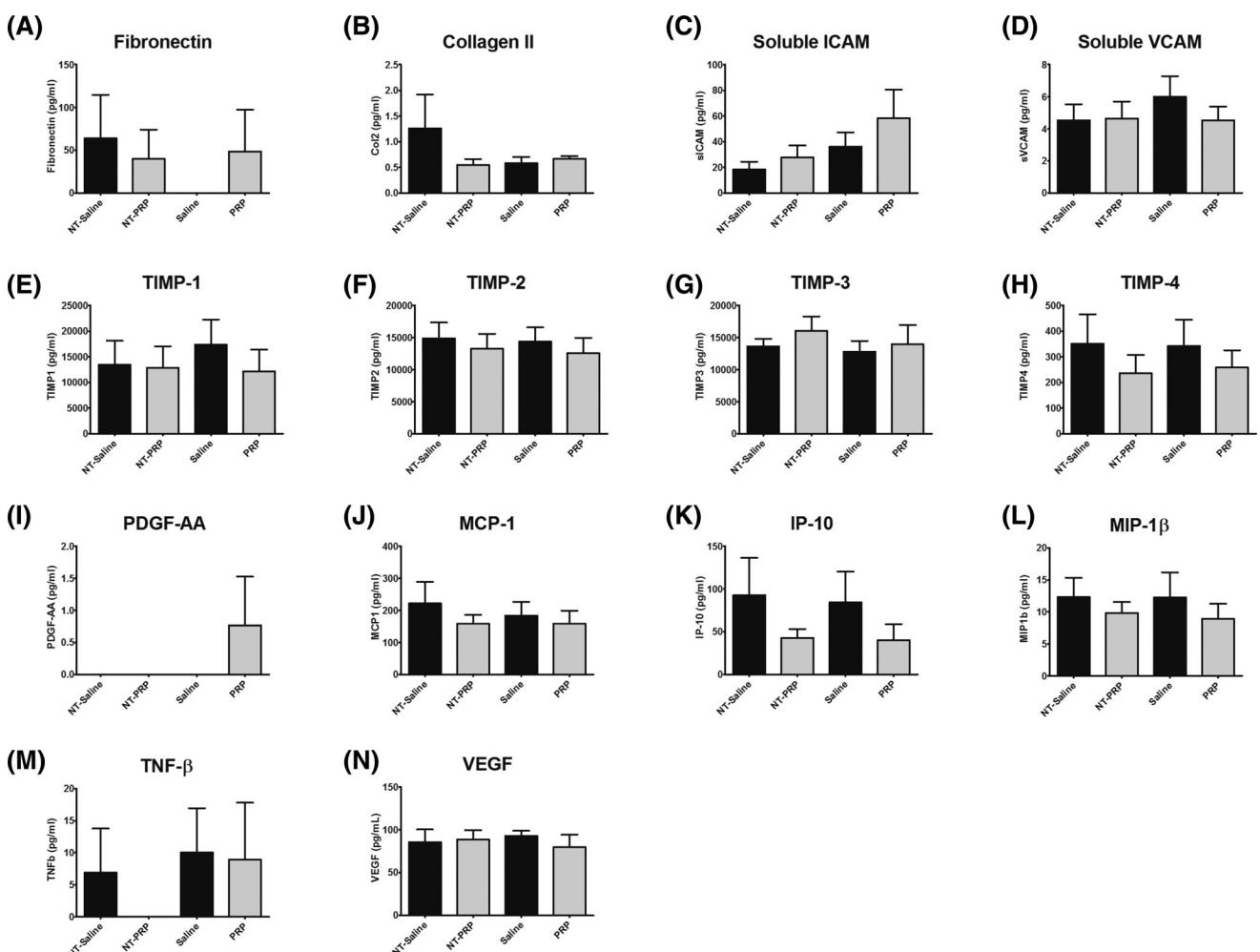
## SF MSC Isolation and Characterization

The pelleted cell fraction was resuspended in phosphate buffered saline and passed through a 70- $\mu$ m filter to remove acellular debris. Cells were plated on tissue culture dishes and cultured in Dulbecco's Modified Eagle Medium supplemented with 5% activated thrombin PRP. Cells were incubated at 37°C, 5% CO<sub>2</sub> for 1-3 days to select for adherent cells. Cultures were not passaged and collected for phenotyping using flow cytometry and gene expression analysis. Adherent cultures were characterized by flow cytometry relying on CD45 and CD31 as negative markers and CD105, CD29, CD73, and CD90 as positive markers for MSCs. To assess trilineage differentiation capabilities, adherent cultures were triggered to differentiate along adipogenic, osteogenic, and chondrogenic lineages, according to standard protocols.<sup>33,34</sup> Briefly, cells were washed twice with phosphate-buffered saline, fixed

in 4% paraformaldehyde for 2 hours at room temperature, and washed twice with phosphate buffered saline. MSCs were then stained with Alizarin Red S (osteogenic), Oil Red O (adipogenic), and Safranin O or Alcian Blue/Glacial Acetic Acid (chondrogenic).

## Gene Expression Analysis in Synovial MSC/Synoviocytes

Quantitative real-time polymerase chain reaction (PCR) was conducted on synovial MSCs using TaqMan chemistry as previously described.<sup>35</sup> cDNA was prepared using the Superscript cDNA Synthesis System. Probes and primer sets were obtained from Applied Biosystems (Foster City, CA). Cyclophilin A (PPIA) was used as an endogenous control. Experiments were performed in duplicate.



**Figure 3. Analysis of ECM and angiogenic biomarkers following PRP treatment.** Biomarker levels were assessed by multiplex ELISA in synovial fluid aspirate prior to and 10 days following PRP or saline treatment. No treatment (NT) indicates baseline levels for the cytokine prior to the patient receiving saline or PRP treatment. One way analysis of variance with Tukey multiple comparison post-hoc test ( $P$  values  $< .05$  were considered significant). ECM = extracellular matrix; ELISA = enzyme-linked immunosorbent assay; ICAM = intercellular adhesion molecule; MCP-1 = monocyte chemoattractant protein-1; MIP-1 $\beta$  = macrophage inflammatory protein-1 beta; PDGF = platelet-derived growth factor; PRP = platelet-rich-plasma; TIMP = tissue inhibitors of matrix metalloproteinase; TNF- $\beta$  = tumor necrosis factor beta; VEGF = vascular endothelial growth factor.

### Quantitative Protein Biomarker Analysis

Millipore MILLIPLEX MAP magnetic bead panels were used for the analysis of human knee SF content. The following panels were used: Human Cytokine/Chemokine Panel II (Cat. # HCYP2MAG-62 K), Human Angiogenesis/Growth Factor Magnetic Bead Panel 1 (Cat. # HAGP1MAG-12 K), Human Cardiovascular Disease (CVD) Magnetic Bead Panel 2 (Cat. # HCVD2MAG-67 K), Human Skin Magnetic Bead Panel (Cat. # SKINMAG-50 K), Human Cytokine/Chemokine Magnetic Bead Panel (Cat. # HCYTOMAG-60 K), Human TIMP Magnetic Bead Panel 2 (Cat. # HTMP2MAG-54 K), Human MMP Magnetic Bead Panel 1 (Cat. # HMMP1MAG-55 K), and Human High Sensitivity T Cell Magnetic Bead Panel (Cat. # HSTCMAG-28SK). All tests were performed following the manufacturer's protocol. Experiments were performed in duplicate. Briefly, samples were diluted, and antibody-bound magnetic beads were added to the wells where they bind to proteins within the SF. All plates were incubated overnight and analyzed on a Luminex MAGPIX multiplexer with xPONENT software. An ELISA (MyBioSource, San Diego, CA, Cat. # MBS018738) was used to analyze the levels of type II collagen in the SF.

### Statistical Analysis

Data are presented as mean  $\pm$  SEM with analysis done using GraphPad Prism 6.0 (GraphPad, La Jolla, CA). For numerical data, analysis comparing two groups (saline vs PRP) used an unpaired Student's *t*-test. For ordinal data collected through WOMAC, analysis comparing more than two groups used a Kruskal-Wallis test with Dunn's post-hoc multiple comparison test. Analysis of WOMAC data included a paired Student's *t*-test that compared only two groups (baseline v. time point). In addition, a nonparametric correlation (Spearman) was used to produce a correlation matrix with *r* values and *P* values to indicate whether improved WOMAC scores at different follow-up time points correlated with respective treatments (Figure S2). The closer an *r* value was to one the stronger the correlation, and *P* values demonstrate whether those strong correlations were significantly different from baseline. Finally, analysis comparing more than two groups of numerical data used one-way analysis of variance (ANOVA) with Tukey multiple comparison post-hoc test. All *P* values  $< .05$  were considered significant.

### Results

#### Patient Screening and Adverse Events

For this pilot study, 28 patients were assessed for eligibility based on the established criteria outlined in the Methods section. At the initial evaluation, six participants were found to have no synovial joint effusion and were excluded. Another five participants declined to participate. The enrollment process occurred from October

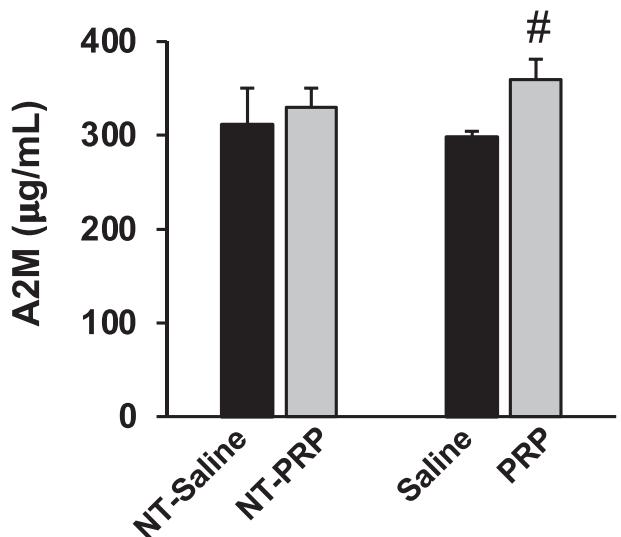
to November 2014 and is summarized in Figure 1. Table 1 highlights the demographics and baseline clinical parameters of all patients participating in the study. One female was excluded 6 months into the study owing to pregnancy; and therefore, she was not included at the 12-month follow-up. One patient developed a hemarthrosis and had this aspirated from the PRP-treated knee joint. On further follow-up this patient had walked greater than 2 miles immediately after the second SF aspiration and overuse was suspected. This patient was seen again in clinic and their pain was at a minimal level several days following aspiration.

### PRP Formulation

A summary of the complete blood counts for peripheral blood and PRP are provided in Table 2. On average white blood cell counts were reduced by greater than 50% and platelet concentrations increased by greater than 100% (Table 2). A representative analysis of selected proteins contained in the PRP samples are presented in Figure S2.

### Analysis of SF Inflammatory and Tissue Remodeling Protein Biomarkers and MSC Gene Expression during PRP Treatment

Cytokines that were detected in the SF of patients receiving saline or PRP are shown in Figure 2. Additionally, IL1 $\beta$ , IL2, and IL4 were analyzed from the SF but did

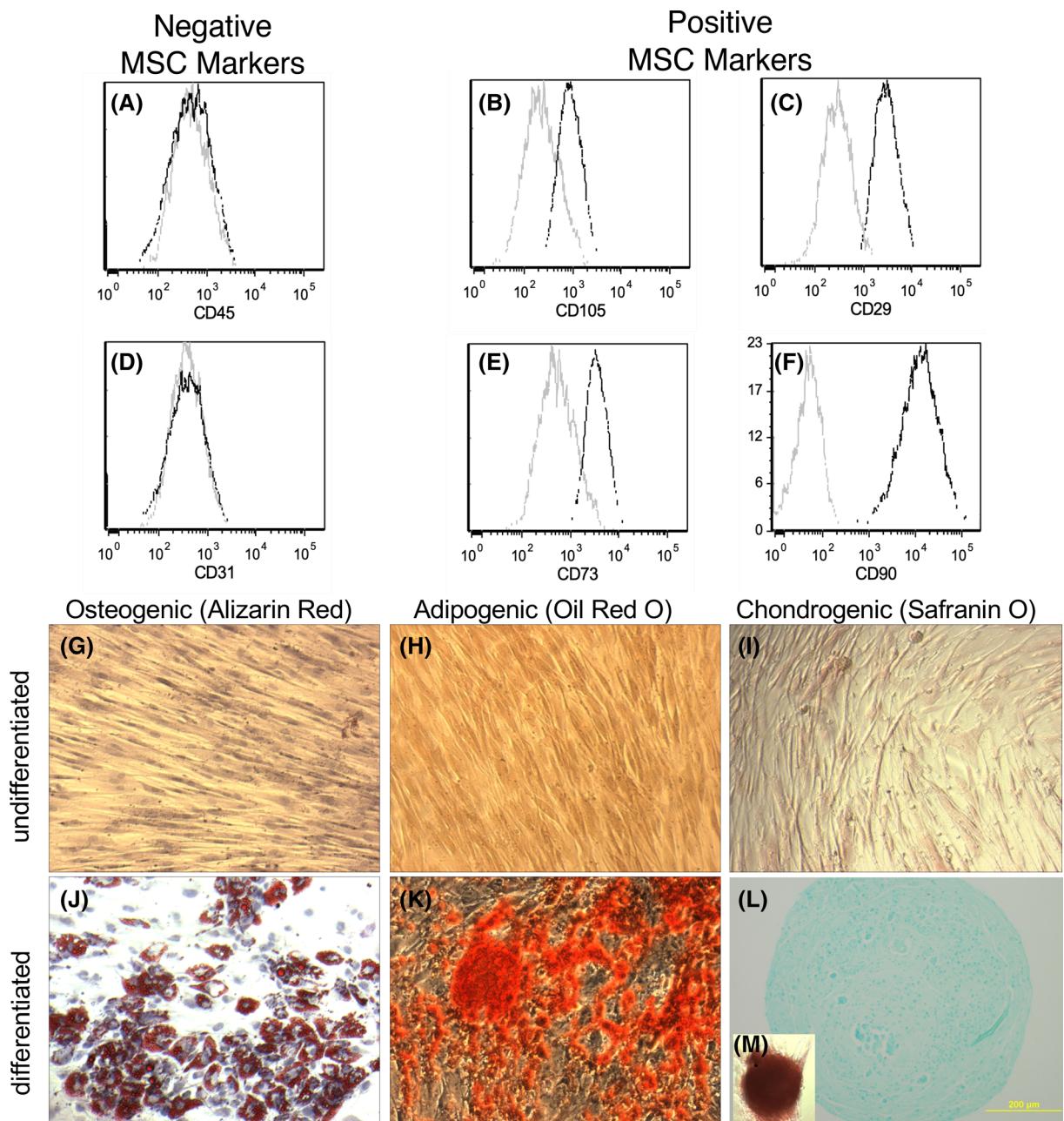


**Figure 4.** Analysis and A2M following PRP treatment. A2M levels in the synovial fluid were assessed by ELISA before and following treatment with saline or PRP. No treatment (NT) indicates baseline levels for the cytokine prior to the patient receiving saline or PRP treatment. Changes were detected in the PRP group following the intervention (#, *P* = .005). One way analysis of variance with Tukey multiple comparison post-hoc test (*P* values  $< .05$  were considered significant). A2M = alpha-2-macroglobulin; ELISA= enzyme-linked immunosorbent assay; PRP = platelet-rich plasma.

not produce a signal that was above the assay detection limit. At 10 days following saline and PRP treatment, we observed moderate changes in IL5, IL6, IL8, IL10, and TNF- $\alpha$  (Figure 2A, B, D, E, G).

We examined the presence of a subset of proteolytic enzymes, extracellular matrix (ECM) proteins, and their inhibitors in the SF of saline- and PRP-treated groups. As shown in Figures 2 and 3, we detected the presence of

several ECM proteins and factors related to ECM turnover. We did not observe changes in the levels of these factors in the SF 10 days following saline or PRP treatment. We assessed changes in factors that regulate the angiogenic response during PRP treatment. We did not observe changes in the set of angiogenic factors that were analyzed at baseline and 10 days following PRP treatment (Figure 3). A2M levels were analyzed in the SF during



**Figure 5. Cell surface marker and trilineage differentiation analysis of synovial mesenchymal stem cells.** (A-F) Mesenchymal stem cells (MSCs) were isolated from the synovial fluid aspirate of patients undergoing saline or platelet-rich plasma (PRP) treatment. Cells were cultured and screened for negative (CD45, CD31) and positive (CD105, CD29, CD73, CD90) MSC markers by flow cytometry. MSCs were tested for their ability to differentiate into three mesenchymal lineages. (G, J) Alizarin Red S stain was used to detect calcium production, which is representative of osteoblast differentiation. (H, K) Oil Red O stain was used to highlight lipid formation indicative of adipocytes. Alcian blue stain (I, L) and Safranin O stain (M) were used to detect glycosaminoglycan (GAG) protein production indicative of chondrogenic differentiation. Unpaired Student's *t*-test (*P* values < .05 were considered significant) was used to assess saline and PRP treatments.

PRP treatment. Treatment with PRP was positively associated with significantly elevated levels of A2M in the SF (Figure 4).

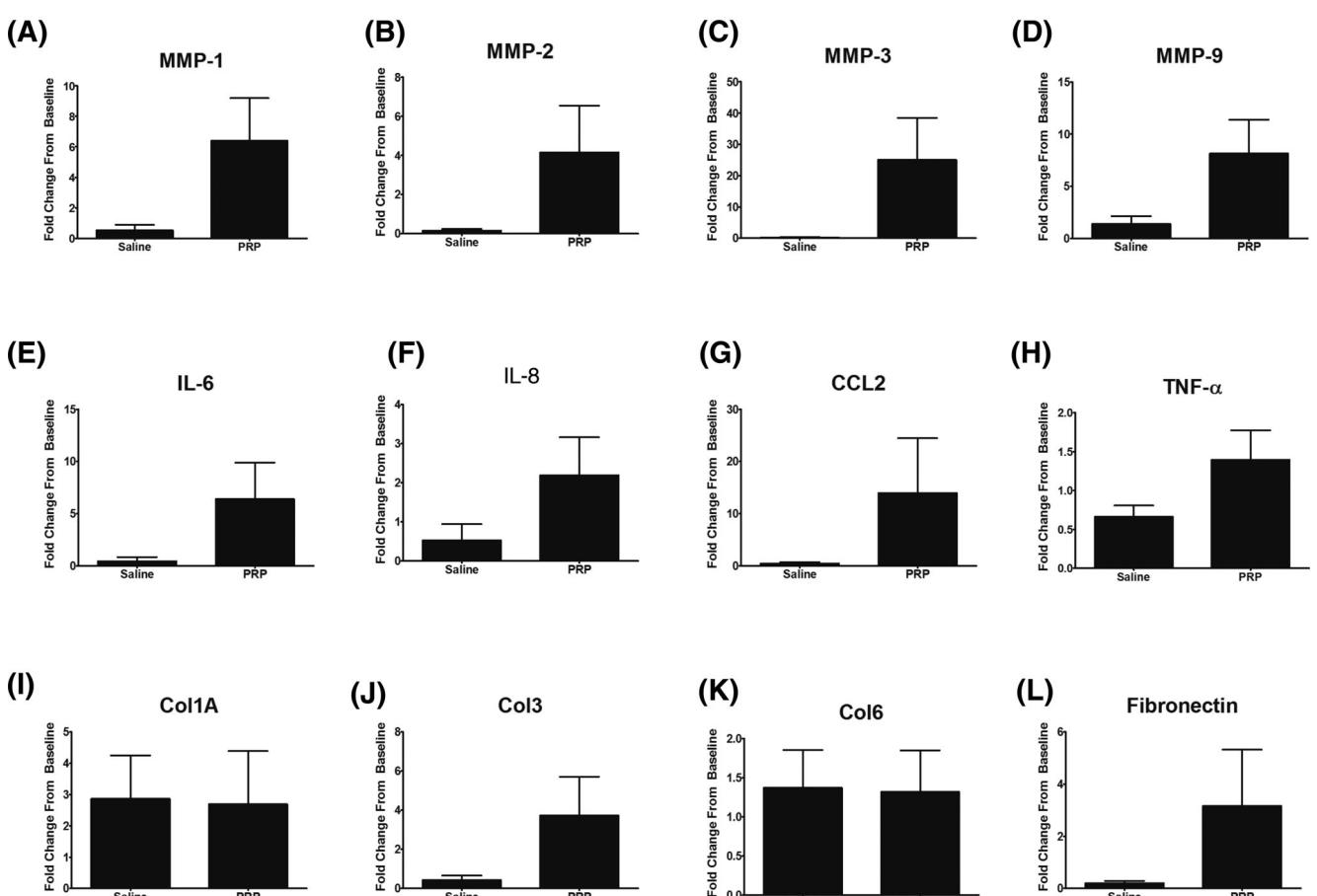
MSCs were isolated from the SF of saline- and PRP-treated patients based on marker expression (Figure 5A-F) adherence to tissue culture plastic, and trilineage differentiation. Cells were minimally passaged in order to retain their phenotypic features. MSCs from saline- and PRP-treated groups demonstrated osteogenic, adipogenic, and chondrogenic potential based on in vitro differentiation protocols and subsequent staining with Alizarin Red (Figure 5G, J), Oil Red O (Figure 5H, K), and Safranin O (Figure 5I, L, M).

Gene expression was first measured using real-time quantitative PCR to determine whether PRP treatments influenced inflammation and extracellular matrix remodeling (Figure 6). We observed a potential association between PRP treatment and an increase in gene expression for all MMPs and several inflammatory markers (IL-6, CCL2, TNF- $\alpha$ ) in synovial MSCs (Figure 6A-H). Collagen 3 and fibronectin mRNA levels were elevated in the

PRP group relative to controls, whereas mRNA expression of Col1A and Col6 were not statistically different than levels measured in controls (Figure 6I-L).

### Knee Radiographs

Knee radiographs were taken prior to beginning the study to confirm the level of osteoarthritis in the knee joint. Fifteen of the 17 individuals had a repeat knee radiograph taken at approximately 6 months following the original injection of either PRP or saline (control). The two participants who did not follow up were in the treatment (PRP) group. In total, all six saline control participants were found to have unchanged knee radiographs from baseline to 6-month follow-up. Of the nine PRP treatment group participants, three showed very slight progression of joint space narrowing. Interestingly, one of the PRP participants was found to have a 0.5-mm increase (improvement) in the medial joint space (data not shown).



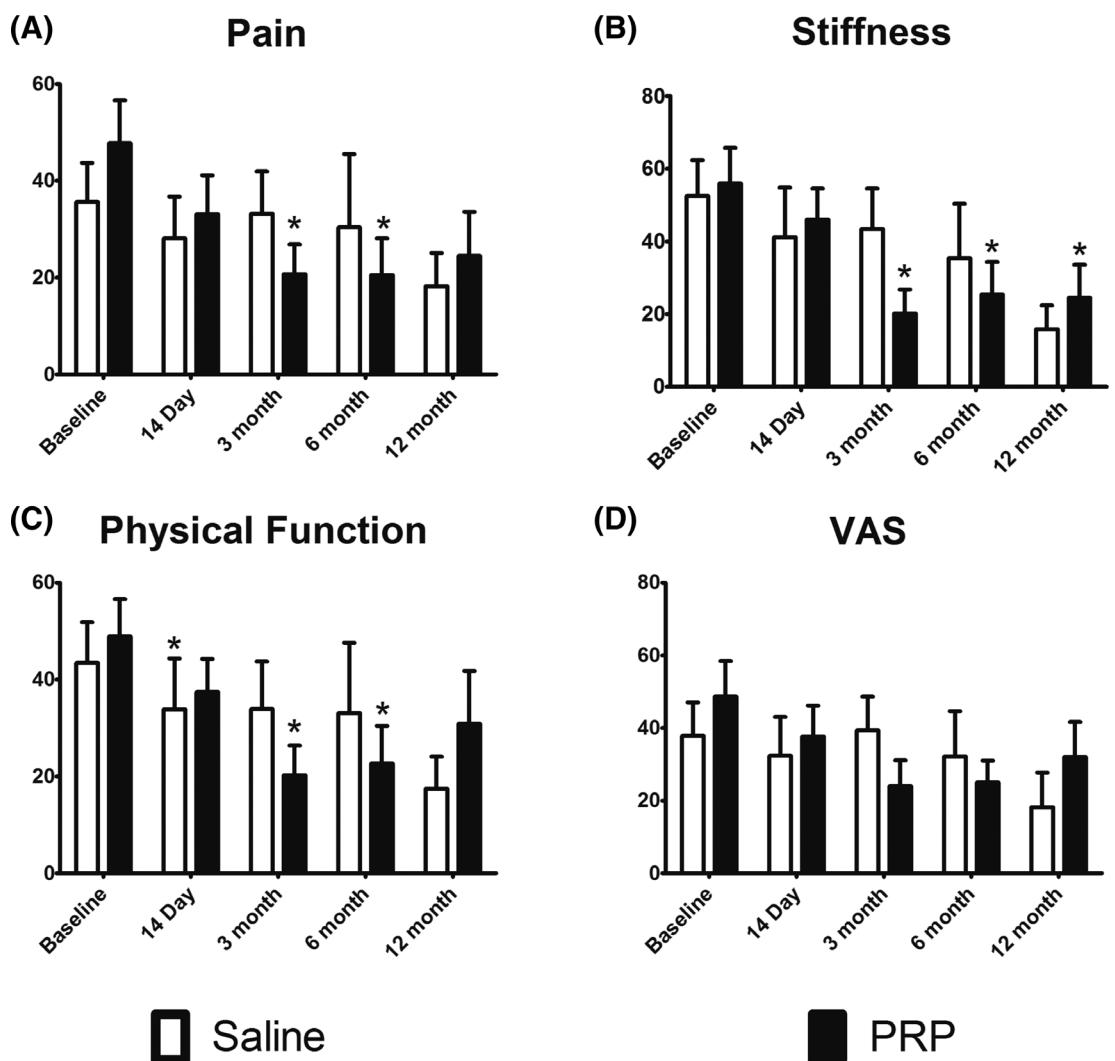
**Figure 6. Overall change of immunomodulatory and tissue remodeling biomarkers following PRP treatment.** Quantitative real-time polymerase chain reaction was used to evaluate ECM degradation biomarkers (A-D), inflammatory biomarkers (E-H), and ECM production biomarkers (I-L). These levels were assessed in synovial fluid aspirate 10 days following PRP or saline treatment. Although average relative mRNA levels were elevated in PRP-treated synovial fluid, no statistical differences were found. Unpaired Student's *t*-test (*P* values < .05 were considered significant) was used to assess saline and PRP treatments. ECM = extracellular matrix; IL = interleukin; MMP = matrix metalloprotease; PRP = platelet-rich-plasma; TNF- $\alpha$  = tumor necrosis factor alpha.

### Clinical Outcomes of Saline (Placebo) and PRP Treatment

WOMAC scores were assessed at several time points over a 12-month period (Figure 7). WOMAC scores were first compared using a Kruskal-Wallis test with Dunn's multiple comparison post-hoc test and no differences were detected. When WOMAC scores were isolated to compare only time points to baseline (paired *t*-test), differences were detected. WOMAC scores declined up to 3 months from initial PRP treatment and remained low at 6 and 12 months. In contrast, WOMAC scores for participants receiving saline treatments were unchanged through 12 months after treatment for pain, stiffness, physical function, and VAS. More specifically, knee pain and stiffness subscores decreased

dramatically from baseline at 3 months post treatment and remained low up to 6 months from treatment (Figure 7A, B). Knee stiffness subscores in participants receiving saline did not decrease until 12 months following treatment, but those data were not found to be significant.

Physical function subscores improved 2 weeks after treatment (Figure 7C). Scores reached statistical significance for PRP-treated knees at 3 months and remained improved at 6 months. In contrast, saline-treated knees were unchanged from the 2-week point until 12 months following treatment, when physical function improved to levels equivalent to those treated with PRP. Although the PRP-treated group trended toward a reduction in VAS at 3 and 6 months, the levels did not reach statistical significance (Figure 7D).



**Figure 7. Mean Western Ontario and McMaster (WOMAC) and visual analog scale (VAS) scores on 100-point scale.** WOMAC scores were assessed over a period of 12 months in both saline- and PRP-treated patients. For the WOMAC and VAS higher scores for pain, stiffness, and physical function are associated with increased pain, stiffness, and difficulty, respectively. Baseline levels were established between treatment groups and WOMAC scores were recorded for (A) pain, (B) stiffness, (C) physical function, and (D) visual analog score. Changes were detected from baseline within saline- and PRP-treated patients, where PRP improved pain, stiffness, and physical function by 3-month intervals compared to baseline (\*,  $P < .05$ ). Kruskal-Wallis with Dunn's multiple comparison test was used to assess differences amongst saline and PRP treatments over time while a paired Student's *t*-test was used to assess baseline versus follow-up time points.  $P$  values  $< .05$  were considered significant. PRP = platelet-rich plasma.

Correlation tests have been previously used to assess WOMAC data.<sup>36,37</sup> We used nonparametric correlation tests and determined that lower scores at 3, 6, and 12 months correlated with saline or PRP treatment for pain, stiffness, physical function, and VAS (Table S2). Incidence of significant correlations appeared to be more frequent at 6 months following treatment for both saline and PRP when compared to baseline.

## Discussion

Studies have shown short-term improvement in pain and function following PRP injections for knee osteoarthritis.<sup>38,39</sup> However, there are few studies investigating the biological effect(s) of PRP on the synovial joint. Chen et al demonstrated that multiple PRP injections reduce total SF volume, protein concentration, and markers of inflammation.<sup>40</sup> The goal of this pilot study was to demonstrate feasibility of quantitatively assessing changes in the synovial microenvironment during PRP intervention and provide insights on the biochemical responses that PRP elicits in the osteoarthritic joint. The dosing of PRP for the treatment of KOA is variable and we report an average total platelet load of  $\sim 3.5 \pm 1.5$  billion platelets for injection (Table 2). Although few clinical studies report the total amount of platelets delivered, our platelet load is similar to several reports.<sup>32,41</sup> We observed a statistically significant increase in A2M in SF retrieved from PRP compared to control group. A2M is known to bind to proteases (such as MMPs) and slow degradation of cartilage such as in osteoarthritis.<sup>42</sup> A2M has gained interest for its potential use to treat musculoskeletal conditions in which joint degeneration is a result of excessive proteolytic activity.

Protein biomarkers were measured using a multiplex system to determine if differences in SF occurred with PRP treatment. Despite a limited sample size, we noted slight changes in protein levels in the early phase following PRP treatment (10 days), including a significant change in expression for A2M. Interestingly, none of the SF samples demonstrated a measurable value for markers associated with OA progression such as MMP-13 or IL-1 $\beta$ . Treatment alone, resulted in moderate increases in TNF- $\alpha$ , IL-5, IL-6, IL-10, and soluble intercellular adhesion molecule. In light of this pilot study, these candidate molecules warrant additional follow-up studies.

We were unable to show symptomatic improvement in the time frame in which biomarkers were analyzed. This may explain why the screen did not uniformly reveal extensive molecular changes within the knee synovial environment. The focus of this pilot study was on molecular changes that occur within the study time frame and we focused our effort on the early phase response. Changes in response to PRP treatment may be delayed more than 2 weeks or require repeated injections.<sup>40</sup> Notably, WOMAC scores, knee stiffness, physical function, and pain scores improved up to 6 months in the

PRP group when compared to the saline group; however, clinically relevant differences in the overall WOMAC scores and knee stiffness were not observed until after the 2-week follow-up. Furthermore, differences in the number of participants for saline and PRP groups and a lower baseline WOMAC might also explain some of the differences observed between the treatment groups. More studies may include SF analysis over an extended period of time combined with WOMAC data correlation to ascertain whether molecular changes affect the joint microenvironment at later time points.

The synovium undergoes extensive changes during osteoarthritis and typically displays an inflammatory pathology. PRP can regulate these inflammatory processes through cytokine and growth factor release. Cellular constituents of the synovium respond to PRP and initiate the healing cascade. Synovial MSCs regulate joint architecture in part by expressing MMPs in order to remodel the ECM, migrate, and stimulate differentiation and proliferation.<sup>43,44</sup> This restorative cell population increases within the synovial fluid in a wide range of musculoskeletal disorders.<sup>19,20</sup> The exact mechanism of this cellular activation is unclear but likely associated with MSC activation during the disease response. In this study, MSCs isolated from the PRP-treated patient population demonstrated increased expression of several key MMPs. These findings are consistent with published data suggesting that synovial MSCs, as well as MSCs derived from other sources, exhibit increased MMP expression in response to PRP.<sup>45-47</sup> The impact of increased MMP expression in synovial MSCs following PRP treatment is unclear and our data preliminarily suggests a reevaluation of the perceived universally negative catabolic effects of MMPs during KOA.<sup>48-50</sup> Additional studies examining changes in the synovium in response to PRP and other orthobiologics may shed light on how these therapeutics influence the healing response during osteoarthritis.

A limitation for this pilot study is that we did not correlate joint or clinical responses with biomarkers within the PRP itself. We also did not consider clinical changes within the synovial space as an indicator of disease improvement. A larger cohort of patients may allow for this type of analysis. The small sample size limits the power of this pilot study focusing on the effects of PRP at a molecular and cellular level on the osteoarthritic joint and our ability to account for multiple testing. Many variables play a role in the characteristics of PRP including a patient's age and gender, and further research could focus on response time as well as sex- and age-dependent changes.

A key consideration for the analysis of cellular and molecular biomarkers is the need to collect an adequate amount of SF from arthritic knee joints. When participants returned for follow-up, there was a variable amount of effusion present. Prior studies have demonstrated a significant decrease in SF effusate following PRP administration.<sup>40</sup> If biomarkers are to be assessed at later time points following PRP injection, strategies for

sufficient SF sample collection need to be considered including saline infusion. In this pilot study we used urea concentration as a strategy to correct for the dilution in order to determine SF volume.<sup>32</sup>

There are ongoing investigations focused on more advanced autologous biological modalities including stem cell products (adipose and bone marrow) for use in osteoarthritis treatment.<sup>51-55</sup> For example, stem cells and bone marrow derived PRP represent new and innovative approaches in biorestorative orthopedics. Our anecdotal experience with these products indicates they are more effective for longer periods of times compared to PRP. Future studies may benefit from looking at the cellular and molecular microenvironmental effects of stem cell derived products on the synovial joint with or without PRP.

## Conclusion

This work offers insights into the cellular and molecular changes that can occur during intra-articular PRP administration in the knee synovial environment and highlights the need for a reevaluation of factors considered to be key drivers of osteoarthritis progression. We demonstrate that the analysis of SF and MSCs of the synovial microenvironment following PRP injections and/or other biorestorative modalities, is feasible and may provide valuable insights into the biological effects of these treatment options.

## Acknowledgments

This publication is dedicated in memory of the late Dr. Jeff Erickson, a leader in the field of cellular and regenerative medicine. Funding for this study was provided by the Foundation for PM&R, the McGuire Research Institute (Investigator funds, LG), and the New Investigator Award from the Richard Materson Education Research Fund of Foundation for Physical Medicine and Rehabilitation. Supplies were donated from the Cytonics Corporation.

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

## References

- Martel-Pelletier J, Barr AJ, Ciccuttini FM, et al. Osteoarthritis. *Nat Rev Dis Primers*. 2016;2:16072. <https://doi.org/10.1038/nrdp.2016.72>.
- Bhatia D, Bejarano T, Novo M. Current interventions in the management of knee osteoarthritis. *J Pharm Bioallied Sci*. 2013;5(1):30-38. <https://doi.org/10.4103/0975-7406.106561>.
- Esser S, Bailey A. Effects of exercise and physical activity on knee osteoarthritis. *Curr Pain Headache Rep*. 2011;15(6):423-430. <https://doi.org/10.1007/s11916-011-0225-z>.
- Buckwalter JA, Anderson DD, Brown TD, Tochigi Y, Martin JA. The roles of mechanical stresses in the pathogenesis of osteoarthritis: implications for treatment of joint injuries. *Cartilage*. 2013;4(4):286-294. <https://doi.org/10.1177/1947603513495889>.
- Robinson WH, Lepus CM, Wang Q, et al. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. *Nat Rev Rheumatol*. 2016;12(10):580-592. <https://doi.org/10.1038/nrrheum.2016.136>.
- Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. *Nat Rev Rheumatol*. 2010;6(11):625-635. <https://doi.org/10.1038/nrrheum.2010.159>.
- Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum*. 2012;64(6):1697-1707. <https://doi.org/10.1002/art.34453>.
- Goldring MB, Goldring SR. Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. *Ann N Y Acad Sci*. 2010;1192:230-237. <https://doi.org/10.1111/j.1749-6632.2009.05240.x>.
- Jia Z, Liu Q, Liang Y, et al. Repair of articular cartilage defects with intra-articular injection of autologous rabbit synovial fluid-derived mesenchymal stem cells. *J Transl Med*. 2018;16(1):123. <https://doi.org/10.1186/s12967-018-1485-8>.
- Koshino T, Wada S, Ara Y, Saito T. Regeneration of degenerated articular cartilage after high tibial valgus osteotomy for medial compartmental osteoarthritis of the knee. *Knee*. 2003;10(3):229-236.
- Shahid M, Kundra R. Platelet-rich plasma (PRP) for knee disorders. *EFORT Open Rev*. 2017;2(1):28-34. <https://doi.org/10.1302/2058-5241.2.160004>.
- Sanchez C, Bay-Jensen A-C, Pap T, et al. Chondrocyte secretome: a source of novel insights and exploratory biomarkers of osteoarthritis. *Osteoarthritis Cartilage*. 2017;25(8):1199-1209. <https://doi.org/10.1016/j.joca.2017.02.797>.
- Hsueh M-F, Önnérnfjord P, Kraus VB. Biomarkers and proteomic analysis of osteoarthritis. *Matrix Biol*. 2014;39:56-66. <https://doi.org/10.1016/j.matbio.2014.08.012>.
- Honsawek S, Yuktanandana P, Tanavalee A, Saetan N, Anomasiri W, Parkpian V. Correlation between plasma and synovial fluid basic fibroblast growth factor with radiographic severity in primary knee osteoarthritis. *Int Orthop*. 2012;36(5):981-985. <https://doi.org/10.1007/s00264-011-1435-z>.
- Rübenhagen R, Schüttrumpf JP, Stürmer KM, Frosch K-H. Interleukin-7 levels in synovial fluid increase with age and MMP-1 levels decrease with progression of osteoarthritis. *Acta Orthop*. 2012;83(1):59-64. <https://doi.org/10.3109/17453674.2011.645195>.
- Schmal H, Niemeyer P, Südkamp NP, Gerlach U, Dovi-Akue D, Mehlhorn AT. Pain perception in knees with circumscribed cartilage lesions is associated with intra-articular IGF-1 expression. *Am J Sports Med*. 2011;39(9):1989-1996. <https://doi.org/10.1177/0363546511406851>.
- Driban JB, Balasubramanian E, Amin M, Sitler MR, Ziskin MC, Barbe MF. The potential of multiple synovial-fluid protein-concentration analyses in the assessment of knee osteoarthritis. *J Sport Rehabil*. 2010;19(4):411-421.
- Helmark IC, Mikkelsen UR, Børglum J, et al. Exercise increases interleukin-10 levels both intraarticularly and peri-synovially in patients with knee osteoarthritis: a randomized controlled trial. *Arthritis Res Ther*. 2010;12(4):R126. <https://doi.org/10.1186/ar3064>.
- Jones EA, English A, Henshaw K, et al. Enumeration and phenotypic characterization of synovial fluid multipotential mesenchymal progenitor cells in inflammatory and degenerative arthritis. *Arthritis Rheum*. 2004;50(3):817-827. <https://doi.org/10.1002/art.20203>.
- Jones EA, Crawford A, English A, et al. Synovial fluid mesenchymal stem cells in health and early osteoarthritis: detection and functional evaluation at the single-cell level. *Arthritis Rheum*. 2008;58(6):1731-1740. <https://doi.org/10.1002/art.23485>.
- Bianco P. "Mesenchymal" stem cells. *Annu Rev Cell Dev Biol*. 2014;30:677-704. <https://doi.org/10.1146/annurev-cellbio-100913-013132>.
- Krüger JP, Honcke S, Endres M, Pruss A, Siclari A, Kaps C. Human platelet-rich plasma stimulates migration and chondrogenic

- differentiation of human subchondral progenitor cells. *J Orthop Res.* 2012;30(6):845-852. <https://doi.org/10.1002/jor.22005>.
23. Chen L, Dong S-W, Liu J-P, Tao X, Tang K-L, Xu J-Z. Synergy of tendon stem cells and platelet-rich plasma in tendon healing. *J Orthop Res.* 2012;30(6):991-997. <https://doi.org/10.1002/jor.22033>.
  24. Sekiya I, Ojima M, Suzuki S, et al. Human mesenchymal stem cells in synovial fluid increase in the knee with degenerated cartilage and osteoarthritis. *J Orthop Res.* 2012;30(6):943-949. <https://doi.org/10.1002/jor.22029>.
  25. Lubis AM, Lubis VK. Adult bone marrow stem cells in cartilage therapy. *Acta Med Indones.* 2012;44(1):62-68.
  26. Belmont PJ, Goodman GP, Waterman BR, Bader JO, Schoenfeld AJ. Thirty-day postoperative complications and mortality following total knee arthroplasty: incidence and risk factors among a national sample of 15,321 patients. *J Bone Joint Surg Am.* 2014;96(1):20-26. <https://doi.org/10.2106/JBJS.M.00018>.
  27. Richards MM, Maxwell JS, Weng L, Angelos MG, Golzarian J. Intra-articular treatment of knee osteoarthritis: from anti-inflammatories to products of regenerative medicine. *Phys Sportsmed.* 2016;44(2):101-108. <https://doi.org/10.1080/00913847.2016.1168272>.
  28. Meheux CJ, McCulloch PC, Lintner DM, Varner KE, Harris JD. Efficacy of intra-articular platelet-rich plasma injections in knee osteoarthritis: a systematic review. *Art Ther.* 2016;32(3):495-505. <https://doi.org/10.1016/j.artro.2015.08.005>.
  29. Campbell KA, Saltzman BM, Mascarenhas R, et al. Does intra-articular platelet-rich plasma injection provide clinically superior outcomes compared with other therapies in the treatment of knee osteoarthritis? A systematic review of overlapping meta-analyses. *Art Ther.* 2015;31(11):2213-2221. <https://doi.org/10.1016/j.artro.2015.03.041>.
  30. Park Y, Lee SC, Nam H-S, Lee J, Nam SH. Comparison of sonographically guided intra-articular injections at 3 different sites of the knee. *J Ultrasound Med.* 2011;30(12):1669-1676.
  31. Mei-Dan O, Carmont MR, Laver L, Mann G, Maffulli N, Nyska M. Platelet-rich plasma or hyaluronate in the management of osteochondral lesions of the talus. *Am J Sports Med.* 2012;40(3):534-541. <https://doi.org/10.1177/0363546511431238>.
  32. Raeissadat SA, Rayegani SM, Babaee M, Ghorbani E. The effect of platelet-rich plasma on pain, function, and quality of life of patients with knee osteoarthritis. *Pain Res Treat.* 2013;2013:165967, 1, 7.
  33. Sachs PC, Francis MP, Zhao M, et al. Defining essential stem cell characteristics in adipose-derived stromal cells extracted from distinct anatomical sites. *Cell Tissue Res.* 2012;349(2):505-515. <https://doi.org/10.1007/s00441-012-1423-7>.
  34. Lu T-J, Chiu F-Y, Chiu H-Y, Chang M-C, Hung S-C. Chondrogenic differentiation of Mesenchymal stem cells in three-dimensional chitosan film culture. *Cell Transplant.* 2017;26(3):417-427. <https://doi.org/10.3727/096368916X693464>.
  35. Zhao M, Sachs PC, Wang X, et al. Mesenchymal stem cells in mammary adipose tissue stimulate progression of breast cancer resembling the basal-type. *Cancer Biol Ther.* 2012;13(9):782-792. <https://doi.org/10.4161/cbt.20561>.
  36. Das Gupta E, Ng WR, Wong SF, Bhurhanudeen AK, Yeap SS. Correlation of serum cartilage oligomeric matrix protein (COMP) and interleukin-16 (IL-16) levels with disease severity in primary knee osteoarthritis: a pilot study in a Malaysian population. *PLoS One.* 2017;12(9):e0184802. <https://doi.org/10.1371/journal.pone.0184802>.
  37. Li L, Li Z, Li Y, Hu X, Zhang Y, Fan P. Profiling of inflammatory mediators in the synovial fluid related to pain in knee osteoarthritis. *BMC Musculoskelet Disord.* 2020;21(1):99. <https://doi.org/10.1186/s12891-020-3120-0>.
  38. Dai W-L, Zhou A-G, Zhang H, Zhang J. Efficacy of platelet-rich plasma in the treatment of knee osteoarthritis: a meta-analysis of randomized controlled trials. *Art Ther.* 2017;33(3):659-670.e1. <https://doi.org/10.1016/j.artro.2016.09.024>.
  39. Shen L, Yuan T, Chen S, Xie X, Zhang C. The temporal effect of platelet-rich plasma on pain and physical function in the treatment of knee osteoarthritis: systematic review and meta-analysis of randomized controlled trials. *J Orthop Surg Res.* 2017;12(1):16. <https://doi.org/10.1186/s13018-017-0521-3>.
  40. Chen CPC, Cheng C-H, Hsu C-C, Lin H-C, Tsai Y-R, Chen J-L. The influence of platelet rich plasma on synovial fluid volumes, protein concentrations, and severity of pain in patients with knee osteoarthritis. *Exp Gerontol.* 2017;93:68-72. <https://doi.org/10.1016/j.exger.2017.04.004>.
  41. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. *Am J Sports Med.* 2013;41(2):356-364. <https://doi.org/10.1177/0363546512471299>.
  42. Cuéllar JM, Cuéllar VG, Scuderi GJ. α2-macroglobulin: autologous protease inhibition technology. *Phys Med Rehabil Clin N Am.* 2016;27(4):909-918. <https://doi.org/10.1016/j.pmr.2016.06.008>.
  43. Mannello F, Tonti GAM, Bagnara GP, Papa S. Role and function of matrix metalloproteinases in the differentiation and biological characterization of mesenchymal stem cells. *Stem Cells.* 2006;24(3):475-481. <https://doi.org/10.1634/stemcells.2005-0333>.
  44. Almalki SG, Agrawal DK. Effects of matrix metalloproteinases on the fate of mesenchymal stem cells. *Stem Cell Res Ther.* 2016;7(1):129. <https://doi.org/10.1186/s13287-016-0393-1>.
  45. Altaie A, Baboolal TG, Wall O, Jones E, McGonagle D. Platelet lysate enhances synovial fluid multipotential stromal cells functions: implications for therapeutic use. *Cytotherapy.* 2018;20(3):375-384. <https://doi.org/10.1016/j.jcyt.2017.12.003>.
  46. Amable PR, Teixeira MVT, Carias RBV, Granjeiro JM, Borojevic R. Mesenchymal stromal cell proliferation, gene expression and protein production in human platelet-rich plasma-supplemented media. *PLoS One.* 2014;9(8):e104662. <https://doi.org/10.1371/journal.pone.0104662>.
  47. Browning SR, Weiser AM, Woolf N, et al. Platelet-rich plasma increases matrix metalloproteinases in cultures of human synovial fibroblasts. *J Bone Joint Surg Am.* 2012;94(23):e1721-e1727. <https://doi.org/10.2106/JBJS.K.01501>.
  48. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. *Front Biosci.* 2006;11:529-543. <https://doi.org/10.2741/1817>.
  49. Meszaros E, Malemud CJ. Prospects for treating osteoarthritis: enzyme-protein interactions regulating matrix metalloproteinase activity. *Ther Adv Chronic Dis.* 2012;3(5):219-229. <https://doi.org/10.1177/2040622312454157>.
  50. Thorson C, Galicia K, Burleson A, et al. Matrix Metalloproteinases and their inhibitors and proteoglycan 4 in patients undergoing Total joint Arthroplasty. *Clin Appl Thromb Hemost.* 2019;1076029619828113:25. <https://doi.org/10.1177/1076029619828113>.
  51. Im G-I. Clinical use of stem cells in orthopaedics. *Eur Cell Mater.* 2017;33:183-196. <https://doi.org/10.22203/eCM.v033a14>.
  52. Pascual-Garrido C, Rolón A, Makino A. Treatment of chronic patellar Tendinopathy with autologous bone marrow stem cells: a 5-year-followup. *Stem Cells Int.* 2012;2012:1-5. <https://doi.org/10.1155/2012/953510>.
  53. Pettine KA, Suzuki RK, Sand TT, Murphy MB. Autologous bone marrow concentrate intradiscal injection for the treatment of degenerative disc disease with three-year follow-up. *Int Orthop.* 2017;41(10):2097-2103. <https://doi.org/10.1007/s00264-017-3560-9>.
  54. Hernigou P, Delambre J, Quiennec S, Poignard A. Human bone marrow mesenchymal stem cell injection in subchondral lesions of knee osteoarthritis: a prospective randomized study versus contralateral arthroplasty at a mean fifteen year follow-up. *Int Orthop.* 2021;45(2):365-373. <https://doi.org/10.1007/s00264-020-04571-4>.
  55. Hernigou P, Bouthors C, Bastard C, Flouzat Lachaniette CH, Rouard H, Dubory A. Subchondral bone or intra-articular injection of bone marrow concentrate mesenchymal stem cells in bilateral knee osteoarthritis: what better postpone knee arthroplasty at fifteen years? A randomized study. *Int Orthop.* 2021;45(2):391-399. <https://doi.org/10.1007/s00264-020-04687-7>.

## Disclosure

**J.D.T.** iOrthoBiologix, Charlotte, NC; and Department of Physical Medicine and Rehabilitation, Virginia Commonwealth University Health System, Richmond, VA.  
Address correspondence to: J.T.; e-mail: drtucker@jobx.com

**L.L.G.** Spinal Cord Injury and Disorders Service, Hunter Holmes McGuire Veterans Affairs Medical Center, Richmond, VA

**M.B.D.** iOrthoBiologix, Charlotte, NC

**J.B.G.** Department of Physical Medicine and Rehabilitation, Virginia Commonwealth University Health System, Richmond, VA

**L.W.E.** American Cancer Society, Atlanta, GA

**S.A.S.** Department of Biomedical Engineering, Parks College of Engineering, Aviation, and Technology, Saint Louis University, St. Louis, MO

**M.J.M.** Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA

**P.V.Q.** Department of Radiology, Hunter Holmes McGuire Veterans Affairs Medical Center, Richmond, VA

**C.C.M.** Department of Pathology, Hunter Holmes McGuire Veterans Affairs Medical Center, Richmond, VA

Disclosure: none

The authors attest that patients were enrolled in this study with written consent

Submitted for publication May 19, 2020; accepted January 5, 2021.