

## Efficacy of Mesenchymal Stem Cells (MSCs) enhanced with Platelet Rich Plasma PRP in Osteoarthritis (OA) Treatment

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### Abstract

Osteoarthritis (OA) is one of the most common joint diseases. This disease commonly develops in the weight bearing joints of the lower limbs, such as the knee and hip joints. Osteoarthritis is considered a chronic degenerative disorder that is characterized by a loss of articular cartilage and it causes detrimental effects on the quality of life and functional status. There is no effective therapy available today that alters the pathobiologic course of the disease. Platelet-rich plasma (PRP), which can be easily isolated from whole blood, is often used for bone regeneration, wound healing and bone defect repair. Platelets contain significant amounts of cytokines and growth factors which are capable of stimulating cellular growth, vascularization, proliferation, tissue regeneration, and collagen synthesis. When stem cells are combined with PRP in the presence of GFs, they are able to promote osteogenesis. A growing interest in the area of regenerative medicine, led by an improved understanding of the role of Mesenchymal Stem Cells (MSCs) in tissue homeostasis and repair, has also been recent focused efforts to explore the potential of stem cell therapies in the active management of symptomatic osteoarthritis. Encouragingly, results of pre-clinical and clinical trials have provided initial evidence of efficacy and indicated safety in the therapeutic use of mesenchymal stem cell therapies for the treatment of knee osteoarthritis. Moreover influences of PRP on proliferation, migration, stemness, preservation of MSC immune-modulatory properties and appearance of senescence phenotype have been explored. This review provides deeply knowledge regarding the use of MSCs and PRP for the treatment of osteoarthritis and their application in clinical studies for the future.

**Keywords:** Platelet-rich plasma (PRP); Mesenchymal stem cell (MSC), Growth factors, Knee osteoarthritis.

### Introduction

Osteoarthritis (OA) is a type of joint disease that results from breakdown of joint cartilage and underlying bone.<sup>(1)</sup> The most common symptoms are joint pain and stiffness. Initially, symptoms may occur only following exercise, but over time may become constant. Other symptoms may include joint swelling, decreased range of motion, and when the back is affected weakness or numbness of the arms and legs. The most commonly involved joints are those near the ends of the fingers, at the base of the thumb, neck, lower back, knee, and hips. Joints on one side of the body are often more affected than those on the other. Usually the symptoms come on over years. It can affect work and normal daily activities. Unlike other types of arthritis, only the joints are typically affected. Causes include previous joint injury, abnormal joint or limb development, and inherited factors. Risk is greater in those who are overweight, have one leg of a different length, and have jobs that result in high levels of joint stress.<sup>(2)</sup> Osteoarthritis is believed to be caused by mechanical stress on the joint and low grade inflammatory processes.<sup>(3)</sup> It develops as cartilage is lost and the underlying bone becomes affected. Current accepted medical treatment strategies are aimed at symptom control rather than disease modification.<sup>(4)</sup>

A high volume of research in bone tissue engineering has been devoted to adult stem cells, which can be isolated from tissues such as a bone marrow or adipose tissue. Mesenchymal stem cells (MSCs) have

been identified as the cells which adhere to plastic, lack of expression and absence of the hematopoietic and endothelial markers and their ability to differentiate into adipogenic, chondrogenic, and osteogenic lineages.<sup>(27,28,29)</sup> Adult bone marrow-derived MSCs (BMSCs) have been the focus of most studies due to the inherent potential of these cells to differentiate into various cell types. In the past decade, MSCs have been employed in the regeneration of bone, especially because of its potential to differentiate into an osteogenic lineage, which is of prime importance in the process of bone growth.<sup>(30,31,32)</sup> It is also known to influence the fate of other cells through the process of paracrine signaling thus providing an osteoinductive and osteoconductive environment for the differentiation of other surrounding cells in the associated region.<sup>(33)</sup> Furthermore, it also governs the immune modulatory potential of the neighboring cells through the secretion of prostaglandins.<sup>(34)</sup>

PRP (platelet-rich plasma) was first defined in 2007 as a preparation of platelets present in a small volume of plasma containing a large amount of growth factors (GFs), which is essential for bone growth and regeneration.<sup>(35)</sup> There are more than 15 GFs present in the PRP with the primary ones consisting of platelet-derived growth factor (PDGF), Insulin-like growth factor (IGF) and Transforming growth factor- $\beta$  (TGF- $\beta$ ) along with their isoforms.<sup>(36)</sup> These GFs have their origin in the alpha granules of the platelets (50–80 per platelet).<sup>(37)</sup> However, recent studies have observed not

only the presence of GFs, but also the cytokines, enzymes, proteins, and fibrinolytic and anti-fibrinolytic proteins which are released upon the activation of the platelets through a mechanical or chemical pathway.<sup>(38)</sup> The factors required for activation may include collagen, thromboxane, calcium, magnesium, serotonin, and other platelet aggregating factors.<sup>(39)</sup> Activation leads to an immediate burst of these GFs, thereby leading to the exhaustion of all the factors within 24 h.<sup>(40)</sup> The benefits involved in the application of PRP in the regeneration of bone involve its availability, ease of isolation, good handling and storage properties and its application in the field of bone tissue engineering.<sup>(41)</sup> In addition, it is autologous which eliminates the risk of disease transmission and immune rejection.<sup>(42)</sup>

### Materials and Method

We explored all the published papers in English indexed in Pubmed from 1990-2017, using key words like PRP, PRP in osteoarthritis, MSCs in osteoarthritis, Platelet Rich Plasma and/or Platelet Lysate and/or Platelet Releasate and Mesenchymal Stem Cells. All the review published during this time on Osteoarthritis, PRP and MSCs were also included. The papers quoted in the references of those articles were also explored. The mainstays of therapy include activity modification, conservative pain management strategies, weight loss, and if necessary, replacement of the affected joint,<sup>(5)</sup> which can control symptoms of OA. The possible targets of treating OA include PRP along with MSCs. This paper reviews available reports on the advantages and possibilities of clinical use of platelet-rich plasma together with MSCs in osteoarthritis

**Growth factor content in PRP and PRP help to promote mesenchymal stem cell proliferation and their applicability in medicine:** PRP contains a host of growth factors such as platelet-derived growth factor, transforming growth factor  $\beta$  (TGF- $\beta$ ), vascular endothelial growth factor, basic fibroblast growth factor, insulin-like growth factor, hepatocyte growth factor, and endothelial growth factor.<sup>(43,44)</sup> These growth factors are found in the agranules<sup>(60)</sup> and released upon platelet activation. It is believed that the mitogenic effects of these growth factors<sup>(46)</sup> help PRP to promote mesenchymal stem cell proliferation,<sup>45,46-51</sup> as well as chondrogenic<sup>(52-54,45,48,55)</sup> and osteogenic<sup>(47-50,56,57)</sup> differentiation both in vitro and in vivo. The TGF  $\beta$  family plays a major role in bone and cartilage development. TGF- $\beta$  is expressed in the growth plate and is an important regulator of chondrocyte proliferation and differentiation.<sup>(58)</sup> Platelet-derived growth factor, another one of the growth factors found in PRP, helps chondrocytes to maintain hyaline-like chondrogenic phenotype and induce proliferation and proteoglycan synthesis, and it is a potent chemotactic factor for cells of mesenchymal origin.<sup>(59)</sup>

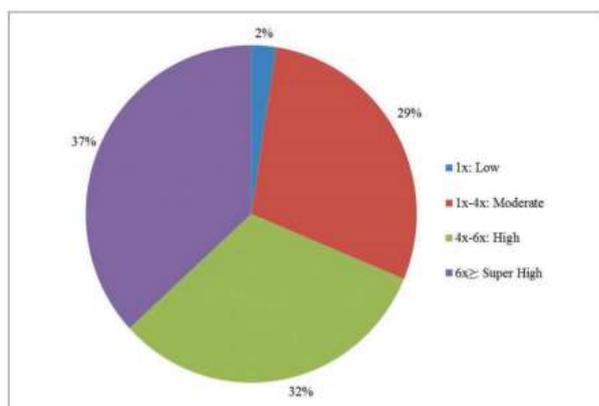
Along with their immunomodulatory and differentiation potential, MSCs have been shown to

express essential cytokines including Transforming Growth Factor beta (TGF $\beta$ ), Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF) and an array of bioactive molecules that stimulate local tissue repair.<sup>(61-63)</sup> These trophic factors, and the direct cell to cell contact between MSCs and chondrocytes, have been observed to influence chondrogenic differentiation and cartilage matrix formation.<sup>(64,65)</sup> Importantly, analysis of mRNA levels within cartilage chondrocytes present at end stage arthritis, indicates that endogenous cells are not inert and remain metabolically active and continue to synthesize cartilage proteins. This supports the hypothesis that MSCs may be able to assist the existing chondrocytes - much like what is observed in their perivascular stromal role within the bone marrow. Indeed, the anti-inflammatory, anti-apoptotic, and anti-fibrotic mechanisms influenced by the properties of MSCs may be their primary mode of activity.<sup>(66)</sup> Autologous MSCs can differentiate into cartilage and bone supporting their potential in the treatment in OA.<sup>(67,68)</sup>

**Preparation of Platelet-Rich Plasma:** Many different procedures for PRP preparation are mentioned in different papers. These all procedures produce different compositions that may account for result variability. Most often the two step centrifugation is used to separate blood components, and to concentrate platelets (and optionally leukocytes) in the final PRP product. Alternatively, one centrifugation step generates a PRP product with a moderate/low concentration of platelets depending on centrifugal force and time (Fig. 1). Overall, it was found that in "PRP+MSC" research there is a slightly higher use of PRPs with high or super high concentrations of platelets (Fig. 2).<sup>(19)</sup>



**Fig. 1: Plasma being removed**



**Fig. 2: Sectors illustrating the use of different PRP formulations in vitro studies of mesenchymal stem cells and PRP**

**Sample collection and MSC expansion:** As mentioned in various articles thirty milliliters of bone marrow were obtained from patients 3–5 weeks prior to injection. Using ficoll hypaque density gradient, the mononuclear cells of bone marrow were separated. Vented flasks (75 cm<sup>2</sup>) with 21 mL MSC medium, consisting of Dulbecco's modified eagles medium (DMEM) with 10% of fetal bovine serum (FBS), were seeded with 1 x 10<sup>6</sup> mononuclear cells (MNCs)/mL for primary culture. Flasks were incubated at 37°C in a humidity chamber containing 5% CO and were fed by complete medium replacement every 4 days, until the confluence of fibroblastlike cells at the base of flasks. Thereafter the adherent cells were re-suspended using 0.025% trypsin and reseeded at 1x10<sup>4</sup> cells/mL. When cells reached confluence by the end of first passage, they were incubated only with M199 medium for one more day. Cells were detached with trypsinization and washed with normal saline supplemented with 2% human serum albumin three times, then resuspended at a density of 1–2 x 10<sup>6</sup> cells/mL.<sup>(14)</sup>

**Injection of MSCs:** After reviewing thoroughly the related articles it is stated that a mean volume of 5.5 mL containing approximately 8–9 x 10<sup>6</sup> cells was prepared and injected in the selected knee of the patient. In each patient, the most painful knee, or the worst knee on

physical examination, was selected as the site of injection. No previous preparation or premedication was given. All antiinflammatory or analgesic drugs were stopped at the entry to the study, 3–4 weeks before the injection of MSCs. Glucosamine was permitted, if the patient was taking it before selection for the study. During the procedure, no joint fluid was aspirated and no steroid was injected in the knee joint. Patients were not hospitalized for the procedure, and went back home half an hour after the procedure. No analgesics, antiinflammatory drugs or immunosuppressive drugs were given or allowed after the procedure.<sup>(14)</sup>

**Proliferation PRP and MSCs large expansion:** The goal of these studies was to establish the best conditions for large-scale expansion of MSCs in cell manufacturing facilities in terms of safety, cost and time. The main goal was to substitute with PRP the xenogenic component of cell expansion, i.e. FBS (fetal bovine serum). These studies primarily calculate cell population doublings through cell passages and population doubling times. They also check MSCs viability at high passages by testing CFU-f (colony-forming unit-fibroblast), phenotype, differentiation capacity and even chromosomal stability and senescence markers. In all the articles, PRP increases the number of cell population doublings, and decreases the time necessary for the population to duplicate. Of note, methodological differences inter studies, including initial cell seeding concentration, MSC passage number conditions, renewal of the medium, and time of the experiment difficult comparisons. Importantly, PRP delays the appearance of the senescence phenotype,<sup>(15,16,17,18)</sup> and protects from chromosomal instability longer than FBS, which has traditionally been used in MSC laboratory cultures.

**Outcome:** PRP preparation techniques varied among studies. Table 1 shows the PRP preparation protocols specific to each study, including PRP system, number of centrifugations, platelet and white blood cell concentrations, and use of an activator (calcium chloride or thrombin).

**Table1: PRP Preparation Protocol**

Study	PRP System	No. of Centrifugations	Mean Concentration (per mL)		Activator
			Platelet	WBC	
Cerza et al. <sup>(6)</sup>	ACP	Single	3-5 (10 <sup>8</sup> )	NR	NR
Filardo et al. <sup>(7)</sup>	Custom	Double	5X WB	1.2 X WB	NR
Kon et al. <sup>(8)</sup>	Custom	Double	>6(10 <sup>7</sup> )	NR	Yes
Spakova et al. <sup>(9)</sup>	Custom	Double	6.8 (10 <sup>8</sup> )	2.3 (10 <sup>7</sup> )	NR
Patel et al. <sup>(10)</sup>	Custom	Double	3.1 (10 <sup>8</sup> )	0	Yes
Li et al. <sup>(11)</sup>	NR	NR	NR	NR	NR

Note: All reported activators were calcium chloride.

ACP, autologous conditioned plasma (Biocore; Arthrex, Karlsfeld, Germany); NR, not reported; WB, whole blood concentrations per injection; WBC, white blood cell.

PRP injection protocol and its application to the joint cavity is mentioned in Table 2 and Fig. 3.



Fig. 3: Application of PRP to joint cavity

Table 2: PRP injection protocol

Study	No. of injections	Injections interval (wk)	Volume per injection (ml)	Injection location
Cerza et al. <sup>(6)</sup>	4	1	5.5	Superolateral
Filardo et al. <sup>(7)</sup>	3	1	5	Not reported
Kon et al. <sup>(8)</sup>	3	2	5	Lateral
Spakova et al. <sup>(9)</sup>	3	1	3	Lateral
Patel et al. <sup>(10)</sup>	2	3	8	Superolateral
Li et al. <sup>(11)</sup>	3	1	3.5	Parapatellar

Leukocyte content influences the molecular composition in PRP. However, most of the in vitro studies don't even mention leukocyte content in their final product. Leukocyte content in PRP samples is merely described by two authors.<sup>(12,13)</sup>

The preliminary report of four patients was mentioned in article related to injection of MSCs. It stated that all four patients were overweight and had their clinical symptoms from 7 to 15 years and so. The different parameters of knee were stated. Firstly the walking time for the pain to appear was 10-20 min. But it improved to 25-60 min. Another was the number of stairs to climb for the pain to appear which approximately was 1-8 stairs. It improved to 20-70 stairs. The next was the time of rest (sitting immobile). It was maximum 15 min. It improved to 30 min after injection of MSCs. On joint examination, the physical parameters improved slightly in comparison to subjective parameters. No patients had instability of knees at baseline and six months follow-up.<sup>(14)</sup>

There is no doubt that any PRP formulation (L-PRP, pure PRP, lysate, releasate) activates MSC proliferation in a controlled non-tumorigenic manner, a property that is of great value not only for cell manufacturing, but also for the clinical applications. Also, PRP is a useful tool to be incorporated in tissue engineering as it acts as a stimulator for cells to proliferate and colonize the scaffold. Table 3 describes the cell culture studies including the proliferation and stemness of MSCs derived from adipose and bone marrow sample.

Table 3: Cell culture studies performed with adipose derived SCs, bone marrow derived SCs

Adipose derived MSCs		
Author/year	Proliferation	Stemness (osteo.)
Chen L. / 201 <sup>(20)</sup>	Enhanced	Enhanced
Chierigato/ 2011 <sup>(21)</sup>	Enhanced	Enhanced
Bone marrow derived MSCs		
Leotot/ 2013 <sup>(22)</sup>	Enhanced	Enhanced
Gottipamula/ 2012 <sup>(23)</sup>	Enhanced	Enhanced
Xia/ 2011 <sup>(24)</sup>	Enhanced	Enhanced
Horn/2010 <sup>(25)</sup>	Enhanced	Enhanced

Chevallier/ 2010 <sup>(26)</sup>	Enhanced	Enhanced
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## Conclusion

Osteoarthritis (OA) is a prevalent chronic degenerative joint disease that will continue to impose an increasing burden on the aging population unless disease-modifying therapies are developed. The current standard of care with risk factor modification, pain management, and joint replacement will be inadequate to meet the needs of society moving forward. Multiple sequential intra-articular PRP injections may have beneficial effects in the treatment of adult patients with mild to moderate knee OA at approximately 6 months. In addition MSCs offer a potential regenerative solution given their ability to differentiate to all tissues within a joint and modulate the local inflammatory response. Although these characteristics suggest they provide ideal building blocks to restore damaged joints, a strong body of evidence supports MSC-guided regeneration through paracrine stimulation of native tissue. Moreover in vitro research in the field "PRP+MSC" need to augment our knowledge and introduces procedural modifications that may help to control the migration, proliferation and differentiation of MSCs for successful healing and for translational purpose.

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