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REVIEW

The Immune-Centric Revolution in the Treatment of Musculoskeletal Disease: Autologous PBMNC and PRP-PBMNC Enriched—A Narrative Review

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ABSTRACT: For over two decades, mesenchymal stem cells (MSCs) have been recognised as the cornerstone of orthobiologic treatments for musculoskeletal diseases. However, clinical evidence increasingly indicates that MSC engraftment in inflamed tissues is minimal and transient, with effects mainly driven by paracrine and immunomodulatory mechanisms induced by macrophage efferocytosis. This evolving paradigm emphasises the immune system as the central orchestrator of tissue repair. Peripheral blood mononuclear cells (PBMNCs) have emerged as potent effectors of regenerative inflammation, mediating apoptotic cell clearance through efferocytosis, facilitating the transition of macrophages from pro-inflammatory (M1) to reparative (M2) phenotypes, and releasing angiogenic and trophic factors that support vascularisation, matrix remodelling, and functional restoration. Clinical studies in critical limb ischemia and diabetic foot provide compelling evidence that autologous PBMNC implantation yields meaningful outcomes in conditions refractory to conventional therapies. Concurrently, platelet-rich plasma (PRP), long regarded as a reservoir of growth factors, is now recognised as a potent recruiter of PBMNCs, where platelet-derived chemokines, such as Monocyte Chemoattractant Protein-1, (MCP-1), Regulated upon Activation, Normal T Cell Expressed and Secreted (RANTES), and Stromal cell-derived factor-1 (SDF-1), establish chemotactic gradients that attract immune cells to injury sites. Neutrophil-depleted, monocyte-enriched PRP formulations demonstrate therapeutic promise in the treatment of osteoarthritis, tendinopathy, and muscle injury. This review consolidates the current scientific rationale and clinical evidence supporting an immune-centric framework, in which PBMNCs, delivered alone or enriched within PRP, constitute a promising next-generation of orthobiologic therapies for musculoskeletal regeneration.

KEYWORDS: Musculoskeletal disease; regenerative medicine; tissue engineering; peripheral blood mononuclear cell (PBMNC); platelet-rich plasma (PRP); mesenchymal stem cell (MSC); translational research; immune-centric revolution; monocytes; macrophages; Treg

1 Introduction

For decades, regenerative medicine has revolved around mesenchymal stem cells (MSCs) as the gold standard for autologous tissue regeneration. The underlying belief was that these cells, when injected into damaged tissues, could directly differentiate and replace injured tissue.

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Caplan et al. [1] redefined MSCs in 2011 as an 'injury drugstore', shifting the field from the differentiation dogma to a paracrine and immunomodulatory paradigm in which trophic factors reprogram macrophages and T cells. Forbes et al. [2] in 2014, and later Julier et al. [3] in 2017, a paradigm shift in regenerative medicine was highlighted, proposing that controlling the immune-mediated mechanisms of tissue repair and regeneration could serve not only to enhance existing regenerative strategies but could represent an alternative to the direct use of stem cells or growth factors.

In the following years, a growing amount of data suggests an additional step toward a more integrated understanding of tissue regeneration—one that recognises the immune system as the master regulator of healing, often referred to as the "immune-centric revolution" or "immune-centric approach" [4–8].

A wide range of immune system components—including inflammatory mediators, immune cells, and cytokines—play a pivotal role in modulating the homeostasis and regenerative capacity of endogenous stem and progenitor cells [9–13]. A deeper understanding of immune–tissue interactions provides the foundation for next-generation regenerative therapies, as strategically leveraging and regulating immune mechanisms may represent one of the most powerful approaches to enhance tissue repair [3–6,8].

It is increasingly evident that immune mechanisms critically shape the regenerative capacity of stem/progenitor cells, highlighting the potential of targeting immune components to enhance therapeutic outcomes and giving rise to the so-called immune-centric revolution, which prioritises the orchestration of local and systemic immune responses over direct stem cell implantation to establish a pro-regenerative environment [4,5,14].

Stem/progenitor cell–based regenerative approaches have achieved limited success across various settings, including orthopaedics and sports medicine applications [15–19]. Autologous MSCs therapies should be considered a second-line injectable option for knee osteoarthritis, as no clear superiority over corticosteroids, hyaluronic acid, or platelet-rich plasma (PRP) has been demonstrated, and their use is recommended primarily when first-line treatments have failed and before surgical intervention is indicated, as reported in the latest European Society of Sports Traumatology, Knee Surgery and Arthroscopy-Orthobiologics Initiative (ESSKA-ORBIT) consensus on cell-based therapy [20]. This underscores a clear unmet medical need and highlights the urgency of developing innovative orthobiologics capable of providing more consistent and effective therapeutic outcomes.

Recent studies converge on the critical role of MSCs apoptosis in driving their therapeutic immunomodulatory function [21–25]: these mounting evidence reveals that the predominant mechanism of action (MoA) of MSCs in inflamed tissues is not engraftment and differentiation, but apoptosis followed by efferocytosis by recruited monocytic cells, leading to macrophage polarization from inflammatory and degenerative M1 to reparative M2 phenotypes [21–25]. Efferocytosis-driven MSCs mechanism of action in inflamed tissue becomes the fulcrum of the immune-centric model: MSCs act as apoptotic immunotherapeutic, with their efferocytosis by inflammatory M1 macrophages triggering a shift to reparative M2 phenotypes, proving that immune cells—not MSCs—are the true effectors of tissue regeneration [21–25].

An impressive amount of data indicates that monocytes, macrophages, and lymphocytes, particularly regulatory T cells (Tregs), representing together the peripheral blood mononuclear cell (PBMNCs) population, are the principal orchestrators of tissue regeneration, both in wound healing and in musculoskeletal tissue regeneration, where their coordinated immunomodulatory activity dictates the balance between inflammation, repair, and functional recovery [26–29].

Unlike MSCs, which are susceptible to apoptosis and show a reduced regenerative potential in inflamed tissues [30–32]. PBMNCs, which provide a heterogeneous immune-stromal repertoire naturally adapted to

circulating and inflamed environments, are emerging as an innovative and promising autologous cell therapy in different clinical settings [33–37].

Interestingly, PBMNCs act not just as passive bystanders but as active drivers of macrophage polarization, shifting the balance from pro-inflammatory M1 to pro-regenerative M2 macrophage [8,38–41] phenotypes, which is a crucial step for resolving inflammation and initiating matrix remodelling. Harvesting and implanting PBMNCs could circumvent the pitfalls of dysfunctional MSCs from chronically inflamed tissues, delivering a clinically feasible, autologous regenerative cell population that directly harnesses immune modulator pathways through monocyte-macrophage crosstalk.

As recently highlighted by Zarubova et al. [6] immune-based regenerative strategies can be deployed through two primary modalities: (i) direct delivery of immune cells like PBMNCs to the lesion site, acting as an autologous cell therapy; or (ii) recruitment and activation of PBMNCs through signals such as those delivered by PRP, which contains growth factors capable of modulating immune behaviour.

This dual strategy—delivery or recruitment—has opened the door to novel therapeutic combinations such as PBMNC [34,37,42,43] or PRP-PBMNC enriched [44–46], which unite the trophic activity of platelets with the immunological intelligence of mononuclear cells.

This review aims to consolidate recent advances in immune-centric musculoskeletal regeneration by: (a) exploring the role of PBMNCs across cartilage, tendon, and muscle healing, (b) highlighting the importance of platelet dose and bioformulation in PRP therapies (c) presenting rationale and preliminary evidence for the synergistic effects of PBMNCs and PRP co-delivery, and (d) framing these strategies as part of a broader move toward precision orthobiologics.

Clinically, PBMNC-based therapies offer a minimally invasive, biologically rational approach that can address both inflammation and tissue degeneration, providing a practical alternative to more invasive stem cell-based adipose- or marrow-derived procedures.

2 Methods

This study is a narrative review. A comprehensive literature search was conducted in PubMed and Scopus from January 2000 to September 2025 using the following keywords: “mesenchymal stem cells”, “MSC”, “peripheral blood mononuclear cells”, “PBMNC”, “platelet-rich plasma”, “PRP”, “immune modulation”, “macrophages”, “musculoskeletal”, “critical limb ischemia”, “diabetic foot”, “osteoarthritis”, “cartilage”, “tendon”, and “muscle”.

Articles written in English were considered. Both preclinical and clinical studies, as well as systematic and narrative reviews, were included.

Case reports, editorials, and conference abstracts were excluded unless complete data were provided. “Complete data” were defined as:

- Reporting of sample size,
- Definition of clinical or experimental endpoints,
- Availability of at least short-term outcomes or follow-up information.

Duplicate or overlapping publications were handled by prioritising the most comprehensive version, defined as the report with the largest sample size, longest follow-up, or most detailed methodology, while earlier or redundant versions were excluded.

Because this is a narrative review, no formal systematic review scoring tools (e.g., JBI, ROBIS) were applied. However, included studies were qualitatively evaluated based on methodological transparency, adequacy of outcome reporting, and relevance to the immune-centric scope. Reference lists were manually screened to identify additional relevant publications.

The primary focus of the review is on the immunomodulatory and regenerative roles of PBMNCs and PRP (including PBMNC-rich formulations) in musculoskeletal disorders.

Fig. 1 was generated with the assistance of ChatGPT, based on an original hand-drawn schematic created by the authors.

3 Stem Cells in Regenerative Medicine—Critical Issue

In orthobiology, MSCs cell–based regenerative therapies have proliferated, yet clinical translation remains modest [15–18,20].

A recent analysis catalogued 449 clinical trials, with only 12.5% having published results, in the domains of osteoarthritis, spinal cord injury, cartilage defects, and tendinopathies, based on the most frequent sources of MSCs: bone marrow (26.5%) and adipose-derived (20.5%); this highlights a significant evidence gap despite intense activity in the field [15].

For example, despite increasing interest in autologous MSCs therapies for knee osteoarthritis, the current clinical evidence remains scarce and heterogeneous [15–18,20]. Small sample sizes, inconsistent protocols, and a lack of long-term or high-quality randomized controlled trials limit most available studies [20]. As a result, there is no clear superiority of autologous MSC injections over established treatments such as corticosteroids, hyaluronic acid, or platelet-rich plasma [15–18,20]. Therefore, the European ESSKA-ORBIT expert consensus recommends that autologous MSC-based therapies should be considered as a second-line option for knee Osteoarthritis (OA), reserved for cases where conventional nonoperative measures and other injectables have failed [20].

In 2023, a significant phase 3 randomized controlled trial involving 480 patients compared autologous bone marrow aspirate concentrate (BMAC), autologous adipose stromal vascular fraction (SVF), and allogeneic umbilical cord tissue-derived mesenchymal stromal cells (UC-MSCs) against corticosteroid injections for knee osteoarthritis [47]. After one year, no treatment arm demonstrated superior pain relief, functional outcomes, or MRI improvements, with all groups showing comparable benefits [47]. Despite cellular heterogeneity in autologous preparations and the presumed potency of allogeneic MSCs, these biological differences did not translate into clinical superiority [47].

From a biological and cellular point of view, this could be due mainly to two critical issues: (a) “real” MSCs are less than expected, and (b) MSC regenerative ability is impaired in inflamed tissue.

3.1 Stem Cells Quantity and Quantification

Recent advances using single-cell transcriptional and proteomic technologies have revealed that the “real” stem cell fraction within clinically applied mesenchymal sources is far smaller than previously assumed [17,47–50].

Ruoss et al. [17] conducted a comparative analysis of BMAC and adipose-derived stromal vascular fraction (ADSVF) obtained from the same patients. Single-cell RNA sequencing, proteomics, and flow cytometry revealed distinct cellular and proteomic profiles: MSCs accounted for approximately 0.22% (±0.22) of nucleated cells in BMAC and about 1.73% in ADSVF. Immune cells dominated BMAC, with significant populations of T cells (approximately 18.7% $CD4^+$ and 5.4% $CD8^+$), natural killer cells (8.8%), monocytes (19.1%), and dendritic cells (6.5%). In comparison, ADSVF was enriched in fibroblasts (about 53.5%), endothelial cells (16.4%), macrophages (10.8%), and pericytes (1.1%) [17]. In conclusion, these data suggest that cell preparations derived from the most popular mesenchymal depots, BMAC and ADSVF, are predominantly composed of T cells, monocytes, and erythroblasts, and fibroblasts and endothelial cells, respectively [17]. Notably, the major contributors to the proteomic content were abundant cellular

populations, including immune cells in BMAC and fibroblasts, macrophages, and endothelial cells in ADSVF, with MSCs and pericytes making minimal contributions [17]. Moreover, many key regenerative factors, such as cytokines, growth factors, and other proteins, were absent or found at low concentrations. [17]. In conclusion, these data suggest that cell preparations derived from the most popular mesenchymal depots, BMAC and ADSVF, are predominantly T cell/monocyte/erythroblast and fibroblastic/endothelial cell injections, respectively [17]. The impact of this observation is particularly striking for adipose tissue, which has long been considered one of the richest reservoirs of stem cells, but is now shown to contain only a minor fraction of true stem-like populations [17].

A recent large randomized trial published by Mautner et al. [47] comparing autologous BMAC, adipose-derived SVF, and allogeneic umbilical cord-derived MSCs (UCT) for knee osteoarthritis revealed no clinical superiority among the cell therapies against corticosteroid controls. The same study showed that single-cell RNA sequencing on the three different cell concentrates demonstrated marked cellular heterogeneity in BMAC and SVF and a more homogeneous MSC phenotype in UCT, highlighting that biological differences in cell composition did not translate into improved clinical outcomes [47].

The findings of Ruoss et al. [17] and Mautner et al. [47] are further supported by several recent studies that apply single-cell analysis to adipose-derived SVF (Table 1). Consistently, independent studies corroborate these findings; Bjerre et al. [48] confirmed that MSC-like cells represent less than 2% of the adipose stromal vascular fraction, showing low inter-donor variability and highlighting the dominant role of immune and stromal heterogeneity. Di Rocco et al. [49] in an extensive review of 17 different studies further demonstrated that bulk tissue analyses overestimate MSC abundance, as single-cell omics reveal rare bona fide mesenchymal progenitors within a complex landscape of diverse subpopulations. Massier et al. [50] identified more than 60 distinct cellular subpopulations within human adipose tissue, underscoring its cellular diversity and confirming MSCs as a minor component.

As shown in Table 1, the proportion of true MSCs varies primarily as a function of the preparation method. Point-of-care centrifugation (e.g., BMAC) enriches hematopoietic and immune cells but yields extremely low MSC frequencies, whereas enzymatic digestion of adipose tissue releases perivascular stromal elements but still results in only modest MSC representation within a highly heterogeneous cell mixture. These methodological determinants are not merely technical details: they critically shape the biological plausibility of stem-cell–mediated repair. The consistently low MSC content observed strongly supports the shift toward immune-centric mechanisms as the principal drivers of musculoskeletal regeneration.

In line with this observation, Veronesi et al. [51] investigated the comparative efficacy of SVF adipose-derived stem cells (ADSCs) and amniotic epithelial cells (AECs) in an experimental sheep model of early osteoarthritis. They reported that SVF treatment resulted in superior preservation of cartilage biomechanics and greater modulation of inflammatory markers when compared to culture-expanded ADSCs [51]. This data highlights that SVF, as a mix of heterogeneous cell populations containing immune and stromal cells, outperforms cultured ADSCs in slowing osteoarthritis progression and improving joint homeostasis. [51]. This suggests that the complex cellular microenvironment within SVF, including immune cells, plays a critical role in tissue repair beyond the contribution of isolated MSCs [51].

Overall, this emerging evidence suggests that classical markers overestimate the abundance of MSCs, with immune and stromal cells—particularly monocytes and macrophages—playing central roles in orchestrating tissue repair, thereby supporting a shift toward an immune-centric regenerative paradigm.

Table 1: Comparative abundance and identity of mesenchymal stem cells (MSCs) and major cell populations in bone marrow concentrate and adipose tissue preparations, stromal vascular fraction (SVF) analyzed by single cell analysis.

Study	Tissue/Source	Preparation Method	% True MSCs	Significant Cell Populations (%)	Notes (Technical Considerations)	Mechanistic Interpretation/Implications
Mautner et al. [47] 71 BMAC, 16 SVF, and 8 UCT-MSC samples at the time of publication were subjected to single-cell RNA sequencing	Bone Marrow (BMAC)	Point-of-care centrifugation EmCyte GenesisCSPure BMAC®-60 mL	~1–3%	Hematopoietic cells, monocytes, lymphocytes	Fresh concentrate; minimal manipulation	Centrifugation enriches immune cells while MSCs remain extremely rare → BMAC cannot plausibly act via stem-cell engraftment; effects likely mediated by monocyte/macrophage activity.
	Adipose Tissue (SVF)	Enzymatic digestion + centrifugation point of care using the enzymatic Transpose RT/Matrase system (InGeneron, Houston, TX, USA).	~1–5%	Immune cells, fibroblasts, endothelial cells	Enzymatic digestion; not point-of-care in USA/EU	SVF remains highly heterogeneous; low MSC purity and high fibroblast contamination limit direct regenerative potential.
	Umbilical Cord Tissue	Allogenic Expanded culture (GMP lab) MSCs are a product of allogeneic cells manufactured from digested umbilical cord tissue that is expanded in culture, cryopreserved and banked.	>80%	Mostly uniform MSC phenotype	Homogeneous MSC population; GMP cultured	Only expanded products achieve high MSC purity, but they are not comparable to clinical point-of-care products.
Ruoss et al. [17] 21 patients BMAC versus ADSVF cell (sub)populations	Bone Marrow (BMAC)	Point-of-care centrifugation Arthrex, Angel BMAC, or EmCyte PureBMC 60 mL bone marrow	~0.022%	Monocytes 19%, T cells >50%	MSCs are extremely rare; an immune-rich product	Confirms that BMAC contains negligible MSCs; any therapeutic effect must derive from immune modulation rather than MSC-driven regeneration.
	Adipose Tissue (SVF)	Enzymatic digestion + centrifugation 90 U of dispase, and 2250 Serine proteinase at 37°C under rotation for 1 h.	~1.7%	Monocytes ~0.68%, T cells <50%, fibroblasts >50%	Very low MSC number; possible contamination by fibroblasts	Low MSC yield + fibroblast predominance undermine stem-cell–centric mechanisms; supports immune-centric interpretation.

Table 1: *Cont.*

Study	Tissue/Source	Preparation Method	% True MSCs	Significant Cell Populations (%)	Notes (Technical Considerations)	Mechanistic Interpretation/Implications
Bjerre et al. [48] 14 patients	Adipose Tissue (SVF)	Enzymatic digestion + filtration Adipose-derived regenerative cells (ADRCs) were isolated from lipoaspirates utilizing a CE-marked, GMP-compliant device (Cytori Celution IV, Lorem Cytori)	<2%	Heterogeneous immune/stromal mix	Rare MSCs; Most of the identified Immune cells are of the myeloid lineage (20% of all ADRC, 71% of all immune cells) T and B cells are the other immune cell populations	Even under optimized enzymatic protocols, MSCs remain < 2%, confirming poor biological plausibility for MSC-driven repair.
Massier et al. [50]	Adipose Tissue (SVF) 50 mg of fresh or frozen White Adipose tissue T was first minced into 1–3 mm pieces and then homogenized on ice.	Enzymatic digestion + centrifugation 50 mg of fresh or frozen White Adipose tissue was first minced into 1–3 mm pieces and then homogenized on ice.	<5% (estimate)	Diverse stromal, endothelial, adipose-derived populations	MSCs are a minority; Adipocytes comprised ~20% of the WAT cell population Immune cells: T, natural killer (NK), and NKT cells (28.8%), and (ii) monocytes, macrophages, and dendritic cells (67.3%), mast cells (2.94%), B (0.87%), and plasma B cells (0.16%).	High heterogeneity dilutes MSC activity; absence of significant MSC enrichment contradicts expectations for stem-cell therapeutic potency.
Di Rocco et al. [49]	Adipose Tissue (SVF)	Enzymatic digestion + single-cell analysis Review of 17 studies	"Small fraction"	Multiple cell types	Single-cell analyses confirm low MSC share Stromal cells, including fibroblast and adipogenic progenitors at different stages of commitment, constituted approximately 40% of the total cell populations, adipocytes were ~20%, immune cells ~20%, and vascular cells less than 15%	Advanced profiling validates that true MSCs represent only a small fraction; supports the immune-centric rationale more strongly than the stem-cell model.

Note: Data compiled from recent single-cell and bulk transcriptomic analyses. Percentages represent the estimated relative abundance of cell populations, including MSCs, immune, and stromal cells. Variations may emerge due to different methodologies and tissue sources. Abbreviation: Adipose-derived regenerative cells (ADRCs), Adipose-Derived Stromal Vascular Fraction (ADSVF), Bone Marrow Aspirate Concentrate (BMAC), Good Manufacturing Practice laboratory (GMP lab), Mesenchymal Stem Cells (MSCs), Stromal Vascular Fraction (SVF), Umbilical Cord Tissue–Mesenchymal Stem Cells (UCT-MSCs), White Adipose Tissue (WAT).

Moreover, considering the minimally invasive nature of peripheral blood collection, the use of PBMNC or PRP derived from peripheral blood offers substantial advantages in terms of patient safety and comfort compared to more invasive harvesting procedures, such as bone marrow aspiration or adipose tissue liposuction. The ease of collection, the possibility of repeating the treatment, combined with a favourable safety profile, represents a key clinical advantage for translational applications.

3.2 Mesenchymal Stromal Cells (MSCs) and Inflammation: Apoptosis as a Therapeutic Mechanism

MSCs have long been considered key players in regenerative medicine due to their multipotency and paracrine effects [1]. Traditionally, MSCs were thought to directly engraft and differentiate into damaged tissues, thereby promoting structural repair, and then exert therapeutic effects through the secretion of bioactive factors [1].

Several studies showed that MSCs are highly influenced by the inflammatory microenvironment [4,5,30,52]. Pro-inflammatory signals ("inflammatory priming") alter their phenotype and immunomodulatory activity, reduce regenerative capacity, impair differentiation, and induce apoptosis, ultimately limiting their survival in inflamed tissues [4,30,52–54]. Pro-inflammatory cytokines not only impair their differentiation capacity but also sensitise MSCs to apoptosis [53], thereby reducing their survival. Eventually, several studies highlight that the key therapeutic mechanism of MSCs in inflamed tissues is their apoptosis, which triggers their engulfment by recruited monocytes, leading to macrophage polarization from pro-inflammatory M1 to reparative M2 phenotypes, rather than direct engraftment or differentiation [21–25] (Fig. 1)

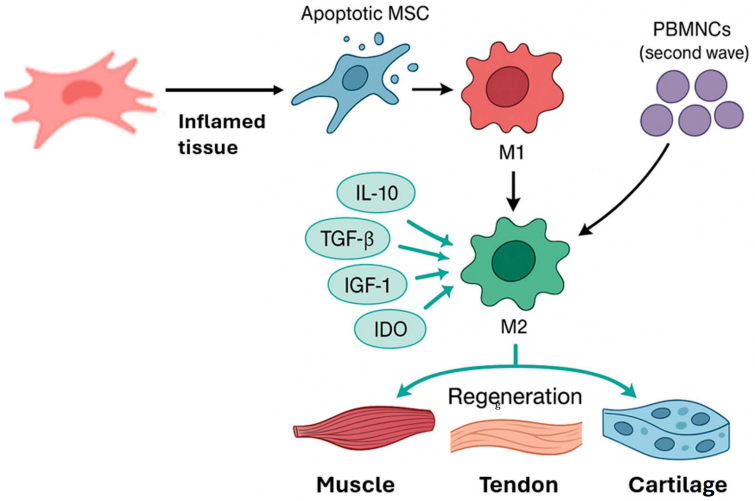

Figure 1: MSCs in inflamed tissue. After administration into inflamed tissues, MSCs rapidly undergo apoptosis. Apoptotic MSCs and their bodies are engulfed by pro-inflammatory M1 macrophages, which are reprogrammed into reparative M2 macrophages. This efferocytosis event induces the secretion of interleukin-10 (IL-10), transforming growth factor-β (TGF-β), insulin-like growth factor-1 (IGF-1), and indoleamine 2,3-dioxygenase (IDO), while suppressing tumor necrosis factor-α (TNF-α) and other inflammatory mediators. In parallel, a second wave of monocytes emerges, consisting of C-X_3-C chemokine receptor 1–positive ($CX3CR1^+$) non-classical monocyte subsets that are recruited by chemotactic signals, reinforcing the M1→M2 transition and sustaining pro-resolving pathways. The resulting M2 macrophages orchestrate angiogenesis, extracellular matrix remodelling, and support regeneration in multiple tissues (muscle, tendon, cartilage) (Created by ChatGPT).

A landmark study by Galleu et al. in 2017 [22] fundamentally altered the understanding of MSC therapeutic mechanisms by demonstrating that MSCs, upon *in vivo* administration, rapidly undergo

apoptosis triggered by the host immune system. This programmed cell death is not merely a passive outcome but a crucial step that leads to a therapeutic immunomodulator. Apoptotic MSCs are engulfed by recipient monocytic cells via efferocytosis, which in turn reprograms pro-inflammatory M1 macrophages into reparative M2 macrophages. [22]. This macrophage polarization initiates a cascade of anti-inflammatory and regenerative signalling—ultimately promoting tissue repair and resolution of inflammation rather than direct tissue replacement by the MSCs themselves [22].

The essential role of MSC apoptosis in therapeutic efficacy has been confirmed and elaborated upon by multiple subsequent studies. Pang et al. [21] showed that apoptosis is a prerequisite for MSC function, with their work providing strong *in vivo* evidence that induction of apoptosis is required to initiate the immunomodulatory cascade. De Witte et al. [23] further elucidated the mechanism, demonstrating that the phagocytosis of apoptotic MSCs by host monocytes triggers functional reprogramming of macrophages toward a reparative profile:

- After administration, MSCs undergo apoptosis and are phagocytosed mainly by a specific non-classical monocyte subpopulation (Ly6C low–expressing monocyte subset).
- The phagocytosis triggers transcriptional and functional reprogramming of these monocytes/macrophages toward an anti-inflammatory M2 profile with increased expression and release of interleukin-10 (IL-10), transforming growth factor-β (TGF-β), insulin-like growth factor-1 (IGF-1), indoleamine 2,3-dioxygenase (IDO) and programmed death-ligand 1 (PD-L1), accompanied by suppression of pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α) These primed monocytes also acquired the ability to induce regulatory T cells *in vitro*, suggesting immunomodulatory effects extending beyond the macrophage population.

Additionally, Vagnozzi et al. [24] and Weiss & Dahlke [25], reinforced that MSC-mediated benefits rely primarily on an acute immune response characterised by macrophage activation and polarization driven by apoptotic cell clearance, rather than MSC survival or differentiation.

Complementing this, Weiss and Dahlke [25] and Watanabe et al. [39] elaborate on the temporal sequence of innate immune responses, describing a first wave of C-C chemokine receptor type 2–positive (CCR2+) pro-inflammatory M1 macrophages followed by a second wave of C-X_3-C chemokine receptor 1–positive ($CX3CR1^+$) non-classical monocytes/macrophages regenerative M2, that mediate inflammation resolution and remodelling.

3.3 Mesenchymal Stromal Cells (MSCs) in Musculoskeletal Inflamed Tissues: Tissue-Specific Constraints of the Apoptotic Mechanism

As detailed in the previous section, the dominant therapeutic mechanism of MSCs in inflamed tissues relies on apoptosis followed by efferocytosis-driven macrophage reprogramming, rather than direct engraftment or differentiation [21–25].

However, in musculoskeletal disorders, this universal mechanism operates within highly inflammatory microenvironments that impose tissue-specific limitations on MSC efficacy. This section focuses on *how musculoskeletal tissues constrain or redirect the outcome of this mechanism*, and why these constraints limit durable regeneration in different musculoskeletal inflamed tissue.

3.3.1 Osteoarthritis Cartilage: Inflammatory Inhibition of MSC Function

In osteoarthritic joints, apoptotic MSCs can modulate synovitis by shifting macrophages from an M1 to a more anti-inflammatory, IL-10/TGF-β-producing phenotype [25]. However, the surrounding environment counteracts sustained repair. OA synovial fluid contains high levels of Interferon-γ (IFN-γ) and TNF-α,

which impair glycosaminoglycan synthesis and disrupt chondrogenic differentiation in both bone marrow- and synovial fluid–derived MSCs [55,56]. As highlighted by Li et al. [32] the inflammatory microenvironment is a major determinant of cartilage degeneration and a key obstacle to effective MSC-based repair.

MSCs encounter a hostile inflammatory microenvironment that markedly reduces their regenerative capacity: inflammatory mediators impair MSC survival, induce aberrant differentiation, and compromise their capacity for tissue repair [31,32,57]. Moreover, the predominance of M1 macrophages within OA synovium further compromises MSC survival and function, reducing their capacity to promote structural matrix restoration [57].

3.3.2 Tendon Disease: Inflammatory Blockade of Tenogenic Lineage Commitment

In tendons, Krishnan et al. [58] demonstrated that exposure to TNF-α irreversibly inhibits the tenogenic differentiation of human MSCs subjected to cyclic mechanical strain, underscoring how inflammation can block lineage commitment. Similarly, Brandt et al. [59] reported that an inflammatory milieu rich in IL-1β, TNF-α, or leukocytes compromises the viability and tenogenic properties of adipose-derived MSCs, leading to impaired scaffold repopulation. Thus, although apoptotic MSCs may still induce macrophage reprogramming, the local biomechanical–inflammatory context prevents meaningful restoration of tendon structure.

3.3.3 Skeletal Muscle Injury: Exacerbation of Inflammation and Fibrosis

In skeletal muscle, Liu et al. [60] showed that bone marrow mesenchymal stem cells (BMMSC) transplantation into inflamed muscle after a contusion exacerbated local inflammation, oxidative stress, and fibrosis, ultimately impairing regeneration. Chazaud et al. [7,61,62] showed that after a muscle injury, inflammation transiently restrains stem cell differentiation, while macrophage efferocytosis and phenotype unlock regeneration, making monocyte/macrophage orchestration indispensable for tissue repair.

Collectively, musculoskeletal tissues impose stringent constraints on the apoptotic immunotherapeutic mechanism of MSCs. Inflammation-driven inhibition of differentiation (cartilage, tendon), misalignment with endogenous repair dynamics (muscle), limit the extent to which efferocytosis-driven macrophage polarization alone can produce durable structural regeneration.

3.4 Mesenchymal Stromal Cells (MSCs) Harvested and/or Implanted in Chronic Inflamed Tissues.

The therapeutic application of MSCs sourced from inflamed tissues—such as adipose tissue in obese or diabetic patients—raises crucial challenges, as their therapeutic potential may be restricted [63].

Obesity exacerbates tissue degeneration and compromises the integrity and reparative potential of MSCs [64,65]. Cells harvested from these hostile microenvironments often bear intrinsic functional impairments, including reduced proliferation, altered differentiation potential, and a senescent, pro-inflammatory phenotype, which collectively diminish their regenerative efficacy when implanted into other inflamed sites, for example, osteoarthritic cartilage [66,67].

In keeping, randomized controlled trials and meta-analyses that have directly compared the efficacy of microfragmented adipose tissue (MFAT) or SVF versus saline or PRP in the treatment of musculoskeletal disorders, especially knee osteoarthritis, did not show a superiority of adipose tissue-based cell therapy [18].

A large randomized controlled trial published in 2025, including 120 patients (60 treated with autologous MFAT and 60 with saline solution), showed that a single injection of MFAT in knee osteoarthritis did not provide statistically or clinically significant benefits compared to placebo up to 24 months [18]. Although slight improvements favoured the treatment group, the between-group difference in Knee Injury and

Osteoarthritis Outcome Score (KOOS) was only 1.7 points—well below the minimal clinically significant difference of 8–10 points [18].

Ye et al. [68] in 2024 conducted a systematic review and meta-analysis that included 4 studies with a total of 266 patients (326 knees) comparing the clinical efficacy and safety of MFAT versus PRP in the treatment of knee osteoarthritis (KOA). Their analysis found that, at 12 months, patients treated with PRP had significantly lower pain scores measured by Visual Analog Scale (VAS) compared to those receiving MFAT, indicating superior long-term pain relief for PRP (mean difference, 0.99; 95% CI, 0.31–1.67; $p = 0.004$). Conversely, at the 6-month evaluation, MFAT resulted in significantly higher Tegner activity scores than PRP, suggesting better short-term improvement in activity function. For other outcome measures, including adverse events, there were no significant differences between the two treatments.

A randomized controlled trial by Baria et al. [69] comparing PRP and MFAT injections in patients with KOA found that both treatments produced clinically meaningful improvements at 6 months, with no significant differences in pain or functional outcome scores, suggesting comparable efficacy between PRP and adipose-derived therapies. In another randomized controlled trial, comparing PRP vs. MFAT for the treatment of KOA, both treatments have demonstrated significant improvement in patient-reported outcomes such as pain relief and function at 12 months post-injection [70]. Interestingly, this study showed that higher Body Mass Index (BMI) negatively impacted the effectiveness of MFAT, while PRP outcomes remained consistent regardless of patient BMI [70]. This suggests a potential advantage of PRP in overweight or obese patients, where MFAT may be less effective.

Zaffagnini et al. [71] conducted a prospective randomized controlled trial comparing a single intra-articular injection of MFAT and PRP in patients with knee osteoarthritis, followed for 2 years. Both treatments led to statistically and clinically significant improvements in pain and knee function scores up to 24 months [71]. Importantly, no significant differences were found between MFAT and PRP groups in clinical outcomes, adverse events, or treatment failures [71]. Radiographic and MRI evaluations showed no disease progression in either group [71]. A minor secondary finding was that a higher percentage of patients with moderate/severe OA in the MFAT group achieved the minimal clinically significant difference for subjective knee scores at 6 months [71]. Overall, the study concluded that a single MFAT injection was not superior to PRP, demonstrating comparable safety and efficacy between these two biological treatments in knee OA [71].

Usuelli et al. [72] showed comparable results to osteoarthritis findings, in a randomized controlled trial in Achilles tendinopathy, confirming that PRP and SVF provide similar improvements in pain and function with sustained efficacy at 2-year follow-up.

In keeping with this data, autologous MSCs cell therapies are positioned as second-line injectable treatments for knee osteoarthritis in the latest ESSKA-ORBIT consensus, given the absence of proven superiority over corticosteroids, hyaluronic acid, or PRP, and should be reserved for cases where first-line interventions have failed, and surgery is not yet appropriate [20].

It is interesting to note that *in vitro* macrophages can inhibit the adipogenic differentiation of human ADSCs primarily through the secretion of pro-inflammatory cytokines, especially TNF-α and IL-1β [73]. Supernatants from M0, M1, and M2 macrophages all exert an inhibitory effect on adipogenesis, with M1 macrophages being the most potent due to higher levels of inflammatory mediators. At the same time, neutralization of TNF-α and IL-1β partially reversed this effect, highlighting their central role in mediating the interaction between macrophages and ADSCs and implicating chronic inflammation as a disruptor of adipose tissue homeostasis and stem cell fate.

In vitro data by Zhang et al. in a similar model showed that human PBMNCs have the opposite effect when co-cultured with macrophages (RAW264.7): PBMNCs significantly inhibit M1 (pro-inflammatory) polarization and promote M2 (anti-inflammatory, regenerative) macrophage polarization [38]. This modulatory action is mediated through the suppression of the STAT1 signalling pathway and the activation of the STAT6 pathway. The study showed reduced expression of M1 markers (such as CD86, TNF-α, IL-1β, and IL-6) and increased expression of M2 markers (like CD206, IL-10, and TGF-β) in both macrophages and PBMNCs. Significantly, monocytes within PBMNCs themselves differentiated into M2-like macrophages under these conditions. This proof-of-concept establishes that PBMNC not only reprograms existing pro-inflammatory macrophages but also actively generates new M2 macrophages, creating an environment that is fundamentally supportive of efficient muscle repair and inflammation resolution [38].

These cumulative studies shift the MSC therapeutic paradigm away from cell replacement toward immunologically orchestrated tissue healing, marking significant progress in both conceptual understanding and clinical translation of regenerative medicine strategies. Collectively, these studies highlight that MSCs act as apoptotic immunotherapeutics, with their efferocytosis by M1 macrophages triggering a shift to reparative M2 phenotypes, indicating that monocytes/macrophages—not MSCs—are the primary effectors of tissue regeneration, particularly in inflammatory environments.

4 PBMNCs Induce Tissue Regeneration through M2 Polarization

While Section 3 highlights the intrinsic limitations of MSC-based therapies—namely the extremely low proportion of true MSCs within BMAC, their poor survival in inflamed or hypoxic tissues, and their propensity to undergo apoptosis or functional exhaustion under inflammatory stress [32,57–59], the following section introduces PBMNCs as the cell population that inherently compensates for these weaknesses.

Unlike MSCs, monocytes and macrophages are evolutionarily adapted to operate within inflammatory and ischemic microenvironments, where they retain viability, metabolic activity, and immunoregulatory competence [74–78].

Their ability to respond to danger signals, orchestrate efferocytosis, and secrete pro-resolutive mediators (IL-10, TGF-β, IGF-1, IDO) allows them not only to survive but to *use* inflammation as a trigger for regenerative programming [23]. This inflammatory tolerance directly addresses the key failure point of MSC therapies, apoptosis in the very tissues they are meant to repair, while their abundance and rapid activation kinetics overcome the quantitative scarcity of MSCs in BMAC.

Taken together, these features establish a clear conceptual shift: PBMNCs do not simply represent an alternative cell source but provide the mechanistic continuity that MSCs lack, enabling an immune-centric model of regeneration grounded in biologic plausibility rather than stem-cell fragility.

Increasing data show that PBMNCs are potent immunomodulators that critically influence macrophage polarisation, driving musculoskeletal tissue regeneration. PBMNCs remain viable after implantation and directly attenuate inflammation by reprogramming macrophages from a pro-inflammatory M1 phenotype toward a pro-resolving M2 phenotype, as shown *in vitro*, *in vivo*, and in clinical trials Table 2.

Building on the *in vitro* data by Zhang et al. [38], Watanabe et al. [39] reported in an extensive review that macrophages are not merely passive responders but active orchestrators of inflammation resolution, dynamically shifting between pro-inflammatory and pro-resolving states through tightly regulated signalling and metabolic reprogramming—mechanisms that underscore the critical role of macrophage plasticity in tissue repair and regeneration.

Table 2: Evidence of M1-to-M2 polarization by peripheral blood mononuclear Cells (PBMNCs) and monocyte-derived macrophages across different tissues.

Author	Model/Clinical Setting	Evidence of M1→M2 Polarization	Key Implications
Zhang et al. [38]	*In vitro*, human PBMC co-culture	PBMC inhibit M1 differentiation and promote M2 phenotype; macrophages enhance M2 polarization of PBMNC monocytes via STAT1↓/STAT6↑	Proof of concept PBMNC immunomodulation M1→M2 *in vitro*
Di Pardo et al. [41]	CLTI, Diabetic foot, wound biopsy (human)	Improved healing; biopsy data suggest ↓M1 ($CD38^+$) and ↑M2 ($CD163^+$)	PBMNC immunomodulation M1→M2 in a diabetic wound correlated with healing
Rehak et al. [8]	CLTI, Diabetic foot (human)	Improved healing; biopsy data suggest ↓M1 ($CD38^+$) and ↑M2 ($CD163^+$)	PBMNC immunomodulation M1→M2 in a diabetic/CLTI patient correlated to healing
Chung et al. [42]	Intervertebral disc degeneration (animal model and clinical trial)	biopsy data: PBMNC promoted a switch in macrophage phenotype from pro-inflammatory M1 to reparative M2	PBMNC treatment markedly preserved disc architecture, enhanced matrix remodelling, and local vascularization
Arnold et al. [40]	Skeletal muscle injury (mouse)	Inflammatory monocytes switch into M2 to support myogenesis	M2 macrophages are essential for muscle regeneration
Misharin et al. [79]	Rheumatoid arthritis (mouse)	Nonclassical monocytes→M2-like macrophages limiting joint inflammation	PBMNC-derived macrophages modulate chronic arthritis
Nahrendorf et al. [80]	Myocardial infarction (mouse)	Sequential recruitment: pro-inflammatory M1→reparative M2 monocytes	Monocyte M1→M2 induce cardiac repair
Shechter et al. [81]	Spinal cord injury (mouse)	Blood-derived macrophages adopt an M2-like phenotype, with an anti-inflammatory role.	M2 macrophages support CNS regeneration.
Schlundt et al. [82,83]	Bone fracture healing (mouse)	Reparative phase, dominated by M2 macrophages	M2 macrophages orchestrate bone repair
Watanabe et al. [39]	Resolution of inflammation	M1→reparative M2 switch as key step for inflammation resolution, tissue regeneration	Conceptual framework for PBMNC immunoregenerative therapy

M1 to M2 polarization induced by the second wave of monocytes in different settings. **Abb.:** Chronic Limb-Threatening Ischemia (CLTI), Cluster of Differentiation (CD), Peripheral Blood Mononuclear Cells (PBMNCs), Signal Transducer and Activator of Transcription 1 (STAT1), Signal Transducer and Activator of Transcription 6 (STAT6), Central Nervous System (CNS).

Preclinical and clinical data demonstrated the polarization ability of PBMNCs in diabetic foot and Chronic Limb-Threatening Ischemia (CLTI) [8,41], skeletal muscle regeneration [40], rheumatoid arthritis [79], myocardial healing [80], spinal cord injury [81], bone fracture repair [82,83] and intervertebral disc degeneration (both animal model and randomized clinical trial) [42].

Chung et al. [42] in 2024 demonstrated that PBMNCs foster the polarization of macrophages from the pro-inflammatory M1 phenotype toward the anti-inflammatory M2 phenotype. This was evidenced by *in vitro* cultures showing increased expression of the M2 marker CD206 on $CD14^+$ monocytes over time, indicating a shift toward M2 differentiation [42]. The *in vivo* component involved a rat intervertebral disc degeneration (IVDD) model created by needle puncture of the caudal discs of Sprague-Dawley rats, wherein human PBMNCs were transplanted intradiscally [42]. Treated rats exhibited attenuated inflammation, preservation of disc matrix components such as aggrecan and type II collagen, and dampened pro-inflammatory cytokines, supporting the immunomodulatory and tissue-reparative roles of PBMNCs through M2 macrophage polarization [42].

Moreover, to explore the polarisation mechanism from M1 to M2, Rodero et al. [84] and Italiani and Boraschi [85] demonstrate that initially, pro-inflammatory "classical" monocytes ($CCR2^+$/$Ly6C^{hi}$) are recruited as a "first wave", differentiating into M1 macrophages responsible for pathogen clearance, debris removal, and production of inflammatory mediators such as TNF-α, IL-1β, and reactive oxygen

species. Subsequently, a "second wave" of anti-inflammatory "non-classical" monocytes ($CCR2^{+}/Ly6C^{low}$) facilitates the reprogramming of M1 macrophages into M2 macrophages through efferocytosis [84,85]. M2 macrophages secrete key factors (IL-10, TGF-β, VEGF) that promote the resolution of inflammation, angiogenesis, and tissue remodelling [84,85]. This biphasic model integrates the essential plasticity and functional specialisation of macrophages, which are critical to effective tissue repair and the restoration of tissue homeostasis.

Without the recruitment of the second wave of anti-inflammatory "non-classical" monocytes ($CX3CR1^{+}/Ly6C^{low}$) and the process of efferocytosis, inflammation becomes chronic, and tissue repair fails. Efferocytosis—the recognition and engulfment of apoptotic cells—serves as a critical driver of the macrophage phenotypic switch, reprogramming pro-inflammatory M1 macrophages into reparative, pro-resolving M2 macrophages. The dynamic alternation and plasticity of these two distinct monocyte waves, through their recruitment into damaged tissue, constitute an indispensable shift from inflammation toward regeneration [8,86].

Overall, all these data suggest that PBMNCs are the key effector cells to induce macrophage polarization into the M2 regenerative phenotype in different tissues and clinical settings.

4.1 PBMNCs: From Bench to Clinics

PBMNCs are emerging as a promising autologous cell therapy in different clinical applications.

Most of the clinical data on PBMNC derive from studies on CLTI and diabetic foot ulcers [87–90]. A large meta-analysis by Rigato et al. [36] demonstrated that autologous PBMNC therapy significantly reduces major amputations in CLTI patients. At the same time, bone marrow mononuclear cells (BMMNC) and BMMSC did not show such a benefit. Additionally, a meta-analysis by Rehak et al. [33] focusing on PBMNCs prepared via selective point-of-care (POC) filtration confirmed improved limb salvage and wound healing outcomes in no-option CLTI patients. Based on this solid clinical evidence, PBMNCs therapy has been indicated in the International Union of Angiology Position Statement on no-option chronic limb-threatening ischemia as the most promising treatment option for no-option CLTI [91].

PBMNC-based therapies have shown safety and efficacy even in chronically inflamed and fibrotic conditions, supporting their broad clinical applicability across distinct pathological conditions [92–96].

Overall, while MSCs currently have broader clinical evidence, the consistent immunomodulatory activity of PBMNCs in various clinical settings, combined with their minimally invasive harvest and proven efficacy in highly inflamed tissues, such as diabetic foot ulcers and critical limb ischaemia, highlights them as a promising alternative or complementary strategy in musculoskeletal regenerative medicine.

4.2 PBMNCs in Osteoarthritis and Cartilage Lesions

Osteoarthritis is characterized by synovial inflammation that drives chondrocyte apoptosis, hypertrophy, ectopic bone formation, and progressive cartilage degeneration [57,97–99]. A recent single-cell atlas of the osteoarthritic knee was published in 2025 [100] revealed a profound shift in the joint microenvironment, with depletion of regenerative tissue stem cells and expansion of "pain-associated macrophages", expressing IL-1β, IL-6, nitric oxide synthase 2 (NOS2), and TNF-α, which cells exhibit an M1-like pro-inflammatory phenotype, directly linking M1-macrophage-driven inflammation to nociception, tissue degeneration, and impaired regenerative potential in OA.

Macrophages within the synovial lining play crucial roles in determining disease progression due to their phenotypic plasticity [99]. Pro-inflammatory M1 macrophages promote cartilage degradation through the secretion of inflammatory cytokines, while anti-inflammatory M2 macrophages support tissue repair

and regeneration by producing factors such as IL-10, IL-1Ra, TGF-β, and ARG-1 [99]. The balance between M1 and M2 macrophages is fundamental for modulating inflammation and promoting cartilage repair, making macrophage polarization a promising therapeutic target in OA treatment [99].

Emerging insights showed that an imbalance of M1/M2 macrophages is linked to the severity level of knee osteoarthritis [57,97,98,101]: chronic synovial inflammation skews macrophages toward a pro-inflammatory M1 phenotype, thereby perpetuating cartilage degradation, pain, osteophyte formation, and impaired chondrocyte homeostasis.

Since MSCs in an already inflamed tissue have impaired properties [25,31,32,57], in addition to therapeutic attempts using MSCs and their derivatives (such as vesicles, microvesicles, and exosomes) for cartilage restoration, inducing M2 macrophage polarization could further enhance the therapeutic effects of MSCs [99].

In vitro studies have demonstrated that M2 macrophages derived from PBMNC induce type II collagen (COL2) expression, a hallmark of healthy articular cartilage, in chondrocytes, establishing a direct link between immune modulation and cartilage matrix regeneration [102]. Human cartilage explants and chondrocyte co-cultures treated with PBMNC show enhanced chondrocyte migration and upregulation of cartilage-specific genes, including collagen type II alpha 1 chain (COL2A1) (gene that encodes for the alpha 1 chain of type II collagen, which is the main collagen found in cartilage) and SRY-box transcription factor 9 (SOX9) (transcription factor crucial for chondrocyte differentiation and cartilage development), highlighting their potential therapeutic benefit in inflamed musculoskeletal tissues [76,103]. Moreover, PBMNC promotes the migration and chondrogenic differentiation of multipotent mesenchymal stromal cells [76,103,104], and the inhibition of synovial macrophage pyroptosis reduces joint inflammation and fibrosis in osteoarthritis models [105]. Direct physical contact between macrophages and chondrocytes is essential for the maturation of regenerative cartilage [102].

Preclinical studies demonstrated that PBMNCs *in vitro* and in an osteochondral defect model stimulate chondrocyte migration, support cartilage repair, and can acquire an MSC-like phenotype under hypoxia, with reparative effects equivalent to bone marrow–derived MSCs [76,103,104].

Yang et al. [106] demonstrated that macrophages derived primarily from circulating monocytes are crucial for spontaneous cartilage repair in an osteochondral injury model, by regulating cell proliferation and apoptosis near the injury site. In adult mice with reduced regenerative capacity, administration of exogenous M2 macrophages promoted cartilage and subchondral bone repair. The regenerative effect depended on the macrophage phenotype and the timing of delivery, highlighting the critical importance of macrophage plasticity in tissue regeneration [106].

A recent study [107] provides compelling histological evidence favouring PBMNCs over PRP for articular cartilage regeneration in a rat model of OA, showing that PBMNC treatment achieved a significant and widespread restoration of collagen fibres, particularly within the non-calcified cartilage zones. Quantitatively, collagen content after PBMNCs injection approached levels observed in healthy cartilage, indicating effective matrix reconstruction [107]. In contrast, PRP treatment elicited only mild and patchy improvements in the collagen network, accompanied by disorganized cellular architecture and hypocellularity [107].

Clinical evidence on the use of peripheral blood–derived autologous cell therapies for cartilage repair is still limited but growing (Table 3). Among the available studies, RCTs provide the highest level of evidence, showing PBMNCs to be superior to HA for both structural and clinical outcomes, whereas observational studies mainly indicate equivalence to PRP, and case series provide feasibility but limited certainty.

Table 3: Clinical trial PBMNC in cartilage disorders/osteoarthritis (OA) studies are ordered by evidence level.

Study	Design (Evidence Level)	Patients (n)	Indication	PBMNC Dose/Source	Comparator	Protocol	Key Outcomes	Evidence
Saw et al. [108]	Case series (Level IV)	5	Chondral lesions	G-CSF–mobilized PBMNCs; ~2.5–4.0 × 10^9 cells (uncultured)	None (single-arm)	5 IA injections weekly + 2 at 6 mo; subchondral drilling + PBMNC + HA	Hyaline-like cartilage regeneration; symptomatic improvement at 24 months	Feasibility and structural repair signal; no comparative efficacy
Turajane et al. [109]	Case series (Level IV)	5	Early knee OA	G-CSF mobilized PBMNCs; ~2.5–5.5 × 10^6 cells/mL	None	Microdrilling + PBMNC + HA + GF	Improved WOMAC/KOOS; safe; 12 mo	Supportive clinical improvement; uncontrolled
Skowroński et al. (2012) [110]	Case series (Level IV)	52	Cartilage defects	G-CSF mobilized PBSC/PBMNC implant	None	Single surgical membrane + PBSC implant	90% good/excellent KOOS/Lysholm; safe	Strong functional signal but uncontrolled
Chiaramonte et al. (2023) [37]	Prospective observational cohort (Level II–III)	16	OA	PBMNC by selective filtration (POC)	Compared with PRP and HA (non-randomised)	1 IA injection	All arms improved; differences modest at 6 months	Suggests equivalence to PRP/HA; no proven superiority
Chuang et al. [34]	Prospective observational (Level II–III)	20	OA	PBMNC, dose 7–12 × 10^6 PBMNC	No true control (PRP/HA co-interventions allowed)	1–3 IA injections	Functional + MRI benefit; safe up to 24 mo	Signals efficacy, but design limits causal inference
Saw et al. [111]	**Randomized Controlled Trial (Level I)**	25 PBMC + 25 HA	Cartilage defects	G-CSF–mobilized PBMNC dose ~3 × 10^7 cells per injection	**HA alone** (randomized)	5 weekly IA injections + 3 at 6 months; subchondral drilling + PBSC + HA	**Improved histology/MRI**, better KOOS; safe at 12 mo	**Highest-quality evidence: PBMNC + HA superior to HA alone**
Turajane et al. [112]	Controlled RCT-like study (Level I–II)	40 PBSC; 20 HA	Early OA	G-CSF–mobilized cells, ~3–5 × 10^6 cells/mL	HA and PRP protocols	3–8 IA injections after microdrilling, PBMNC + HA + GF	No TKA; best pain/KOOS relief in PBMNC arm	Suggests superiority over HA/PRP; moderate quality
Chen et al. [113]	Meta-analysis (Level I–II)	8 studies	Cartilage lesions/OA	PBMNC/$CD34^+$	Heterogeneous (HA, PRP, membranes)	Varies by included study	Consistent efficacy; overall safety acceptable	Highest-level synthesis; evidence consistent across designs

Note: RCTs and the meta-analysis constitute the highest level of certainty. Case series and observational studies provide supportive but lower-certainty data, insufficient to establish comparative efficacy. Abb: IA: Intra-articular injection, GF: Growth Factor (e.g., PRP, G-CSF); HA: Hyaluronic Acid; G-CSF: Granulocyte Colony Stimulating Factor; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; KOO: Knee Injury and Osteoarthritis Outcome Score; KOOS: Knee Injury and Osteoarthritis Outcome Score; $CD34^+$, $CD105^+$: Cell surface markers for stem/progenitor cells; POC: Selective Filtration: A point-of-care selective filtration method for PBMNC, TKA: Total Knee Arthroplasty, OA: Osteoarthritis.

Saw et al. [108] conducted a pioneering case series on evaluating the regenerative potential of PBMNCs combined with hyaluronic acid following arthroscopic subchondral drilling in five patients with articular cartilage defects. At follow-up, all patients exhibited significant clinical improvement, as evidenced by increased Lysholm and International Knee Documentation Committee (IKDC) scores and a reduction in pain levels [108]. Notably, histological analysis of biopsy samples from two patients 24 months after the procedure demonstrated the formation of near-hyaline cartilage, characterised by the presence of mature chondrocytes and matrix organisation closely resembling native articular cartilage [108]. This study provides early clinical and histological evidence that PBMNCs, in combination with hyaluronic acid, can effectively promote high-quality cartilage regeneration in humans with challenging cartilage lesions.

Turajane et al. [109] conducted another case series on five patients with early-stage knee osteoarthritis (Kellgren-Lawrence grade 2–3) refractory to conservative treatment. The patients received three weekly intra-articular injections of autologous mobilised PBMNC, added to PRP and hyaluronic acid (HA), as a post-drilling procedure. PBMNCs were mobilised (through subcutaneous human granulocyte colony-stimulating factor (S-hG-CSF) induced leukocytosis) to enhance homing in the knee joint from the peripheral circulation. Clinical outcomes demonstrated significant improvement in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and KOOS scores at both 1 and 6 months, allowing all pain medications to be withdrawn one month post-treatment. Electron microscopy and histological staining confirmed cell attachment, proliferation, and cartilage matrix formation, indicating the presence of hyaline cartilage. The same group published a randomised controlled trial [112] evaluating the use of mobilised PBMNCs combined with PRP and Hyaluronic Acid (HA) vs. HA alone in patients with early knee osteoarthritis who had not responded to conventional treatments. Sixty patients were divided into three groups: two received PBMNCs with or without growth factor addition, both alongside arthroscopic microdrilling and HA injections, while the third group received only HA. The primary goal was to prevent the need for total knee arthroplasty (TKA), and secondary outcomes included pain, stiffness, and function, as measured by the WOMAC score. Results revealed that none of the patients receiving mobilised PBMNC required TKA within 12 months, while three in the HA-only group did. Additionally, PBMNC groups showed significant improvement in WOMAC scores, with faster symptom relief noted in the group supplemented with growth factors.

Skowroński et al. [110] conducted an observational clinical study involving 25 patients with osteochondral lesions of the knee, graded Kellgren-Lawrence grade 3–4, with defect sizes ranging from 4 to 12 cm^2. Patients received treatment with PBMNCs combined with a modified sandwich surgical technique that included microfracturing, autologous spongy bone grafting from the iliac crest, and collagen membrane application. The PBMNCs were isolated from peripheral blood obtained before surgery and concentrated without *ex vivo* expansion. Follow-up evaluations at 6 months, 1 year, and 5 years demonstrated significant clinical improvement as assessed by KOOS, Lysholm, and VAS scores, along with MRI-confirmed cartilage regeneration and tissue integration. The treatment was associated with safety, sustained functional benefits, and superior cell dosing compared to patients treated with bone marrow concentrate. These findings support the efficacy of autologous PBMNCs therapy combined with surgical techniques for treating substantial osteochondral knee lesions [110].

Similarly, after conducting a preliminary case series study [108], Saw et al. [111] proceeded with a randomised controlled trial involving 50 patients with full-thickness cartilage defects graded as III or IV. The patients underwent arthroscopic subchondral drilling followed by postoperative intra-articular injections of either PBMNCs combined with HA or HA alone. The PBMNCs group received five weekly injections starting one week after surgery, plus an additional three injections at six months post-surgery.

At 18 months, with careful blinding in the histological assessment of cartilage regeneration, histological evaluation of biopsies revealed significantly better cartilage regeneration quality and a greater presence of hyaline-like cartilage in the PBMNC group compared to the HA-only group. Magnetic Resonance Imaging (MRI) at 18 months also indicated superior structural cartilage repair in the PBMNC group. Despite these positive imaging and histological findings, clinical outcome measures such as the IKDC subjective knee evaluation score did not differ significantly between groups at 24 months post-treatment.

More recently, Chuang et al. [34] performed an open-label, single-arm pilot study in 20 patients with moderate-to-severe knee osteoarthritis (Kellgren-Lawrence 3–4), treated with a single intra-articular injection of enriched PBMNCs—containing approximately 8×10^7 cells in 4 mL. Interestingly, the PBMNCs were isolated from 100 mL of peripheral blood and showed an increased proportion of anti-inflammatory M2 macrophages *in vitro* and demonstrated significant anti-inflammatory activity comparable to BM-MSCs in preclinical arthritis models, resulting in significant and sustained improvements in VAS and KOOS scores over 24 months, with no serious adverse events reported. Clinical assessments using the VAS for pain and KOOS resulted in significant and sustained improvements in both scores over 24 months, with no serious adverse events reported.

Recent observational data by Chiaramonte et al. all [37] compared intra-articular injections of HA, PRP combined with HA (PRP-HA), and PBMNCs prepared via a selective filtration POC system in 46 patients with moderate knee osteoarthritis (KL grades II-III). All three treatments demonstrated efficacy in improving pain, joint function, and quality of life over a six-month follow-up period, as measured by validated scales including the VAS, WOMAC, IKDC, and performance-based tests. Notably, PRP-HA and PBMC treatments showed superior improvements in knee range of motion and specific functional scores relative to HA alone. Safety profiles were favourable across groups, with primarily mild and transient local adverse reactions.

Finally, in their comprehensive review, Chen et al. [113] systematically evaluated all available *in vivo* studies investigating the therapeutic efficacy of PBMNC for cartilage defects across both animal models and human clinical trials. The analysis encompassed 130 patients in the eight human studies; 121 achieved significant clinical improvement in cartilage repair. Clinical endpoints included clinical scales (e.g., KOOS, WOMAC, Lysholm, IKDC), imaging (Magnetic Resonance Imaging, X-ray, Computed Tomography), second-look arthroscopy, and histopathology. Surgical implantation and repeated intra-articular injections were both used; cell dosages varied considerably among studies. Histologically, several studies described regeneration of cartilage resembling hyaline cartilage following PBMNCs therapy, and no serious adverse events linked to PBMNC therapy occurred [113].

Within this context, PBMNCs offer a promising immune-regenerative tool that addresses both the inflammatory dysregulation and the failure of cartilage repair characteristic of OA, aiming to enrich and deliver monocytes that will differentiate into M2 anti-inflammatory, pro-regenerative macrophages. These studies suggest that PBMNCs are safe and potentially effective in treating cartilage and osteoarthritis. Despite the small sample sizes and heterogeneous designs, the results are promising and highlight the urgent need for larger randomized controlled trials, ideally including direct comparisons with established autologous stem cell or platelet-rich plasma therapies.

Traditional approaches using MSC have shown variable results in OA due to poor survival in inflamed joints, senescence, and loss of multipotency under oxidative stress, both in preclinical [31,32,57] and clinical studies [18,20,47].

By contrast, PBMNCs are easy to prepare, in particular by a POC selective filtration system [37,114], or in PRP-PBMNC enriched [45], primed to survive and function in inflammatory environments, and immunologically “smart”.

Moreover, PBMNCs can act synergistically with PRP, whose growth factors (PDGF, TGF-β, VEGF) are potent PBMNCs recruiters [6,44,115,116]. In PRP formulations enriched in PBMNCs, mononuclear cells may create a regenerative microenvironment capable of resolving inflammation and triggering chondrogenic repair cascades. [44,45,115,117].

Collectively, these studies demonstrated suggestive clinical improvements in cartilage repair, strong safety profiles, and histological evidence of near-native cartilage regeneration, underscoring the therapeutic promise of PBMNCs in regenerative medicine.

These findings support the potential of cell-based therapies such as PBMCs as a viable, minimally invasive option for symptomatic relief and functional improvement in osteoarthritic knees, warranting further randomised controlled trials to validate long-term efficacy and structural joint effects.

4.3 PBMNCs in Intervertebral Disc Degeneration

Recent studies have revealed a substantial imbalance favouring pro-inflammatory M1 macrophages over anti-inflammatory M2 macrophages within the degenerated intervertebral disc, with M1 macrophages significantly increased in the nucleus pulposus and correlating with the severity of disc degeneration [118]. Notably, Li et al. [119] indicates that elevated M1 macrophage presence in herniated lumbar discs may be therapeutically targeted by promoting polarization towards the regenerative M2 phenotype, highlighting a promising immunomodulatory strategy for disc repair.

PBMNCs activated under the hypoxic microenvironment of the intervertebral disc upregulate crucial regenerative factors, including BMP-2, BMP-6, and Growth Differentiation Factor 5 (GDF5), which have been shown to contribute significantly to the preservation and restoration of disc structure and function [78].

Chung et al. [42] conducted a comprehensive study evaluating the therapeutic potential of PBMNCs for intervertebral disc degeneration, characterised by a hostile microenvironment of chronic inflammation and severe hypoxia, which typically undermines cell survival and tissue regeneration encompassing both preclinical animal models and a pilot clinical trial in humans. In the animal study, enriched PBMNCs were injected intradiscally in an *in vivo* model of disc degeneration [42]. Despite these adverse conditions, PBMNC treatment markedly preserved disc architecture, promoted a switch in macrophage phenotype from pro-inflammatory M1 to reparative M2, and enhanced matrix remodelling and local vascularization, collectively fostering tissue repair and functional recovery [42].

For clinical translation, the same paper's authors performed a randomised trial on 36 patients with chronic discogenic low back pain, divided into three groups: conservative care, leukocyte-poor platelet-rich plasma (LP-PRP), and PBMNC therapy [42]. At six-month follow-up, only the PBMNC-treated group showed significant and sustained improvements in pain (VAS) and disability (ODI), with over 50% response rate in all patients [42]. In contrast, the PRP group did not significantly outperform the conservative care group [42]. MRI evaluations revealed stabilisation of disc degeneration and a reduction in bone marrow oedema exclusively in the PBMNCs group [42]. Although limited by the small sample size, these results suggest superior immunomodulatory and regenerative benefits of PBMNCs therapy compared to leukocyte-poor platelet-rich plasma LP-PRP in the difficult-to-treat environment of intervertebral disc degeneration.

4.4 PBMNCs in Tendon Disorders

Tendinopathy, whether acute or chronic, is increasingly understood not as a purely mechanical overload injury but as a persistent immune-mediated failure of resolution [120,121].

Dean et al. [122] highlighted that immune-mediated inflammatory processes are fundamental mechanisms underlying tendinopathy pathogenesis, extending beyond purely mechanical overload injury.

Subsequently, Jomaa et al. [123] provided a systematic review demonstrating persistent infiltration and activation of inflammatory cells, such as macrophages and lymphocytes, in chronic tendinopathy, which perpetuate tissue pathology due to failure of inflammation resolution.

Arvind et al. [124] further emphasised that while acute inflammation is essential for tissue repair, maladaptive chronic inflammation can lead to tendon degeneration and impaired healing. Russo et al. [125] focused on the dysfunctional synergistic interaction between immune and stem cells as a key factor in the persistence of inflammation and the chronicity of tendinopathy.

Finally, Jiang et al. [126] detailed how an imbalance between type I and type II inflammatory responses creates a critical barrier to tendon–bone healing, with unresolved inflammation driving disease progression. Moreover, recently, single-cell RNA sequencing of human tendinopathic tissue identified a pathogenic $CCL4L2^{+}$ subset of pro-inflammatory M1 macrophages that disrupted the communication between macrophages and tendon stem/progenitor cells, suggesting a mechanism by which sustained and chronic inflammation impairs tendon regeneration [127]. Collectively, these studies support the paradigm that persistent immune activation, characterised by high M1 levels and failure of inflammation resolution, represents key therapeutic targets for tendinopathy management.

The comprehensive review by Sunwoo et al. discusses therapies aimed at shifting macrophage polarisation towards the reparative M2 phenotype, which could improve tendon repair outcomes by reducing fibrosis and chronic inflammation typical of tendinopathy [128].

The *in vitro* study by Yoshida and Murray [129] highlighted the crucial role of PBMNCs in enhancing tendon healing processes. Their experiment employed a model in which human anterior cruciate ligament fibroblasts were cultured within a three-dimensional collagen scaffold, closely resembling the native tendon environment, allowing for the assessment of cellular responses to treatments with PBMNCs, PRP, or both over 14 days. The results showed that PBMNCs significantly boost the anabolic effects of PRP on ligament fibroblasts by promoting increased collagen gene expression, protein synthesis, and cell proliferation [129]. Notably, the regenerative potential of PRP is significantly enhanced by the immunomodulatory actions of PBMNCs, which operate through cytokine signalling pathways, particularly IL-6 release [129]. This synergy between PBMNCs and PRP emphasises the vital role of immune cells in tissue repair and establishes PBMNCs as key effectors in coordinating tendon regeneration.

Preclinical animal studies have been instrumental in elucidating the role of macrophages in tendon healing, recently reviewed [29]. Dakin et al. [130] emphasised the importance of both inflammation and its resolution in tendinopathy, highlighting the therapeutic potential of modulating immune responses. Sugg et al. investigated phenotypic changes in macrophages and the induction of epithelial-to-mesenchymal transition genes following acute tenotomy and repair, revealing dynamic macrophage involvement in tendon regeneration. Meanwhile, macrophage depletion impairs neonatal tendon regeneration in mice, confirming the essential role of macrophages in tissue recovery [131]. Moreover, macrophages educated by extracellular vesicles promote early healing of the Achilles tendon in murine models, emphasising macrophage-mediated regenerative processes [132]. Another study, using a rat model of rotator cuff repair, showed that mechanical stimulation promoted tendon-to-bone healing through an IL-4/JAK/STAT pathway–dependent M2 polarisation [133]. Collectively, these studies highlight macrophages as pivotal orchestrators of tendon healing, with their molecular and phenotypic regulation representing promising therapeutic targets.

Clinical experience with PBMNCs in tendon disorders remains limited. Currently, the only published human evidence is a small case series involving partial Achilles tendon injuries, where ultrasound-guided injection of autologous PBMNCs—obtained at the point of care using a selective filtration system from

100 mL of peripheral blood—led to significant clinical improvement, with mean AOFAS scores rising from 37 at baseline to 82.7 at two months, and MRI scans showing early fibrillar continuity without safety concerns [35].

A narrative review on regenerative medicine applications for foot and ankle disorders emphasised the translational potential of PBMNCs, highlighting their availability at the point of care through selective filtration systems and their immunomodulatory capacity to facilitate angiogenesis and tissue repair [134] underscoring the need for controlled clinical studies to validate their efficacy in tendon and joint pathologies.

Collectively, these findings support the translational rationale for investigating PBMNC-based approaches in human tendon regeneration, as stated by Xu et al. [135] "*Managing macrophage numbers and phenotypes may lead to possible novel therapeutic approaches to the management of early tendinopathy, early acute tendon rupture, hence, promote healing after restoration*". Given their accessibility and immunomodulation function, PBMNCs could represent a next-generation, physiologically compatible, autologous, and minimally manipulated solution for tendinopathies that fail to respond to conservative or standard care.

4.5 PBMNCs in Muscle Regeneration

Muscle regeneration critically depends on a tightly orchestrated immune response requiring coordinated interactions among satellite cells, immune cells, endothelial cells, and fibroblasts [62].

As elegantly summarised by Chazaud, pro-inflammatory M1 macrophages dominate the early phase, ensuring debris clearance and releasing TNF-α, IL-1β, and Reactive Oxygen Species (ROS); however, successful repair requires a timely transition towards an anti-inflammatory M2 profile. M2 macrophages secrete IL-10, TGF-β, and IGF-1, thereby stimulating satellite-cell proliferation and differentiation, while preventing fibrosis [62].

This insight was demonstrated *in vivo* by Ratnayake et al. [136]: regenerative M2 macrophages temporarily form a niche for muscle stem cells by secreting nicotinamide phosphoribosyltransferase (NAMPT), which enhances satellite cell bioenergetics and supports their proliferation and commitment; conversely, the targeted depletion of these macrophages disrupts satellite cell homeostasis and delays or impairs regeneration.

Building on this axis, PBMNCs provide a readily available reservoir of circulating monocytes that can acquire an M2-like phenotype within an appropriate cytokine milieu (e.g., IL-4/IL-13) [137]. After damage, M1 macrophages clear debris and initiate inflammation (TNF-α, IL-1β, ROS), followed by a necessary shift to M2 macrophages that release IL-10, TGF-β, and NAMPT to support satellite-cell proliferation/differentiation, angiogenesis/Extracellular Matrix (ECM) remodelling, and antifibrotic repair [136].

Wosczyna and Rando [138] conceptualized this process as a "muscle stem cell support group": regeneration is not driven by satellite cells alone, but emerges from coordinated interactions with immune cells, fibro/adipogenic progenitors (FAPs), and endothelial cells. Within this ecosystem, macrophages act as central switchers, dictating whether FAPs promote myogenesis or drift towards adipogenesis and fibrosis.

In chronic inflammatory conditions, such as ageing, obesity, or muscular dystrophies such as sarcopenia, this cross-talk becomes impaired [139]. Persistent exposure to TNF-α and IL-6 dampens satellite-cell function, while dysfunctional macrophages fail to complete the M1 to M2 transition and release excessive TGF-β, fostering fibrosis rather than regeneration [139].

The key point is that, within this network, macrophages play a key role in the transition from inflammation to proliferation and remodelling [62,138,140].

When delivered into injured muscle, PBMNCs can (i) recalibrate the inflammatory set-point by shifting macrophage balance towards M2 and reducing TNF-α/IL-1β signalling; (ii) enhance the satellite-cell

compartment through NAMPT-dependent NAD^+ support and IGF-1 paracrine cues; (iii) limit fibrosis and facilitate proper remodelling via IL-10 release and coordinated Matrix Metalloproteases/Tissue Inhibitors of Metalloproteasis (MMP/TIMP) regulation; and (iv) improve revascularisation, as monocyte-derived macrophages promote VEGF-driven endothelial activation and perivascular crosstalk—together accelerating the transition from the inflammatory to the proliferative/remodelling phases of repair [141,142].

In brief, recent evidence indicates that exercise itself can influence immune cell infiltration into skeletal muscle, with acute bouts triggering sequential recruitment of neutrophils and macrophages [143]. The transition from pro-inflammatory M1 to reparative M2 macrophages is crucial for effective regeneration; conversely, chronic conditions are characterised by impaired polarization, a defect that PBMNC-based therapies may help to overcome. This suggests a potential synergy between cell therapy and exercise in promoting muscle repair [143].

Complementing this, Juhas et al. showed in engineered muscle constructs that the inclusion of macrophages enhanced myofibre alignment, vascularisation, and contractile function, emphasising the necessity of macrophage–satellite-cell cross-talk for effective repair [141].

Scala et al. [137] further emphasised that this dialogue must be sustained over time, as its disruption in chronic or fibrotic conditions compromises endogenous regenerative capacity.

Overall, within this framework, PBMNCs—rich in monocytes capable of differentiating into M2 macrophages under appropriate cytokine cues (e.g., IL-4, IL-13)—offer a clinically accessible, point-of-care strategy to restore immune balance, re-establish the regenerative niche, activate satellite cells, suppress fibrosis, and promote angiogenesis through VEGF-mediated endothelial cross-talk.

This mechanistic rationale has been translated into clinical practice: Caravaggio et al. [35] reported a case of severe hamstring tear treated with intralesional PBMNCs prepared by selective filtration, which led to rapid pain reduction, accelerated functional recovery, and MRI evidence of organised muscle regeneration, supporting the feasibility and therapeutic potential of this approach in acute muscle injury.

Although direct clinical data in humans are limited, PBMNCs have shown promise in severe muscle tears and post-surgical recovery (e.g., rotator cuff, hamstring), chronic muscle degeneration, including conditions with immune dysregulation (e.g., post-infectious or autoimmune myopathies), and reconstructive sports medicine, as an adjunct to biologically guided rehabilitation [35,134].

The ability to harvest PBMNCs or PRP enriched with PBMNCs, through a simple blood draw and prepared at the point-of-care, further supports their feasibility, autologous safety profile, and cost-effectiveness in routine clinical practice [44,103,134].

In conclusion, these insights highlight the therapeutic potential of PBMNCs therapy, which may restore the immunological dialogue within the muscle niche, accelerate the resolution of inflammation, and re-establish a regenerative microenvironment. In this view, PBMNCs and PRP-PBMNCs enriched, act not only as cellular effectors but also as “immune modulators”, capable of rebooting the muscle repair program in both acute injuries and chronic degenerative settings.

5 Platelet-Rich Plasma Therapies

PRP is increasingly applied in musculoskeletal disorders, with current evidence highlighting the impact of dosing, leukocyte content, and preparation methods on its therapeutic efficacy.

PRP is not a single product, but rather a category of biologic preparations derived from autologous whole blood [44,144,145]. Its composition and therapeutic efficacy are profoundly influenced by multiple technical and biological factors—particularly the total platelet dose, presence or absence of leukocytes, and standardisation of preparation protocols [44].

Despite over two decades of clinical use, PRP remains highly variable. As reported by Fadadu et al. [146] and Magalon et al. [147], there are more than 50 commercially available PRP systems, each producing products that differ in platelet concentration, leukocyte content, red blood cell contamination, and growth factor levels. These differences are not insignificant: they may explain the variability in clinical outcomes seen across different studies and indications [44]. According to Fadadu et al. [146] not only the definition of PRP is inconsistently applied, ranging from 150,000 to over 1,000,000 platelets/μL concentration, with little agreement on ideal leukocyte content or activation method. PRP must be considered a formulation, not a substance. Reporting guidelines such as Minimum Information for Studies Evaluating Biologics in Orthopedics (MIBO) and DEPA are critical to ensuring data transparency [148].

5.1 Platelet Dose over Concentration in OA

The traditional classification of PRP by platelet concentration (i.e., "2×", "5×", "10×" baseline) is insufficient and misleading; instead, recent high-quality evidence—particularly the systematic review and meta-analysis by Berrigan et al. [149,150] suggests that the absolute platelet dose delivered per injection I sa key predictor of clinical success.

Berrigan et al. [150] identified a consistent threshold of ≥4 billion platelets per injection as the minimum effective dose in knee osteoarthritis. Below this dose, clinical efficacy was negligible. A total cumulative dose of ≥10 billion platelets, administered across multiple injections (three implants), was associated with the most sustained improvements in pain relief, functional recovery, and chondroprotection [44,115,150]. These findings are reinforced by the Dose Efficiency Purity Activation (DEPA) classification proposed by Magalon et al. [147], which recommends reporting not only concentration but also dose, efficiency, purity, and activation.

In knee osteoarthritis, PRP with ≥5–10 billion platelets showed superior outcomes in VAS, WOMAC, and KOOS scores, while PRP doses < 2.5 billion platelets were not significantly better than placebo in multiple RCTs [149–152].

Patel et al. conducted a randomised, triple-blind clinical trial comparing the efficacy of a conventional dose (4 mL) versus a "superdose" (8 mL) of leukocyte-poor PRP, both with the same platelet concentration (~3.5 times baseline; ~700 × 10^3/μL), in patients with early knee osteoarthritis (KL 1–2) [152]. A total of 99 knees were randomised: group A received 4 mL of PRP with approximately 2.8 billion platelets, while group B received 8 mL of PRP with approximately 5.6 billion platelets, with all preparation procedures being identical except for the injected volume and dose [152]. Patients were assessed at baseline, 6 weeks, 3 months, and 6 months using WOMAC, WOMAC Pain, VAS pain, KOOS, and patient satisfaction scores. Both groups showed significant improvement from baseline across all measures; however, the superdose group (8 mL) consistently demonstrated superior improvements in pain reduction and functional scores at every time point [152]. At 6 months, patient satisfaction was notably higher in the superdose group (96% vs. 68%) [152]. Minor, transient side effects such as pain and stiffness were more common with the larger dose, but resolved spontaneously [152]. This high-quality randomised study directly addresses the longstanding debate regarding platelet dose versus concentration in PRP therapy [152]. Using PRP of equal concentration, the authors demonstrated that doubling the volume (and thus the total platelet dose) led to better clinical outcomes. These findings suggest that, for PRP injections in knee osteoarthritis, the total platelet dose delivered—not just concentration—is key to maximising therapeutic benefit. This shift emphasises moving from focus solely on PRP "concentration" to optimisation of both PRP dose and absolute platelet count for improved and sustained pain and function outcomes.

Bansal et al. [151] in a randomised, double-blind controlled trial including 50 patients with moderate OA, patients were randomised to receive a single intra-articular injection of either a high-dose leukocyte-poor PRP (containing 10 billion platelets in 8 mL) or 4 mL of high-molecular-weight HA. Patients were followed for 12 months and evaluated using the WOMAC, IKDC, 6-min walking distance, radiography (joint space width), MRI (cartilage thickness), and measurements of inflammatory cytokines in synovial fluid (IL-6, TNF-α, IL-8) [151]. At all timepoints, PRP significantly outperformed HA for pain reduction, functional improvement, and quality of life [151]. The PRP group showed better WOMAC and IKDC scores, greater pain-free walking distance, and required fewer rescue medications [151]. MRI showed unchanged cartilage thickness in 83% of PRP vs. 62% of HA knees at 1 year. [151]. Synovial fluid cytokine analysis revealed greater reduction in IL-6 and TNF-α at 1 month in the PRP group, correlating with clinical improvements [151].

In a real-world observational data study, De Matthaeis et al. [45] confirmed that three injections of 4 billion platelets in neutrophil-depleted, PRP-PBMNC-enriched plasma achieved 69–74% responder rates at 12 months in Kellgren-Lawrencegrading system (KL) I–III OA patients, with no adverse events and consistent clinical improvement across time points [52]. Cumulative dosing (2–3 injections every 15 days) was necessary to achieve durable effects over 6 months, supporting the concept of 10 billion platelet PRP for OA treatment.

In keeping with a high dose, Nouri et al. [153] found in a study on hip OA that 5–6 mL LP-PRP injections with ≥7 billion total platelets yielded better VAS, WOMAC, and Lequesne scores compared to high molecular weight hyaluronic acid.

Interestingly, to ensure an adequate high platelet dose PRP with minimal manipulation, Prost et al. [154] in a large retrospective real-world cohort (n = 431, follow-up up to 18 months), demonstrated that a single high- or very high-volume injection (≈17 mL, ~9 billion platelets) of pure PRP significantly improved WOMAC and VAS scores across mild to severe knee osteoarthritis. More than half of the patients achieved Outcome Measure in Rheumatology-Osteoarthrtis Research Society International Responder Criteria (OMERACT-OARSI) responder status at 6 months, and efficacy was independent of age, BMI, or radiographic grade. Authors [154] pinpoint that the injected volume and the platelet dose—are critical determinants of long-term clinical benefit.

In a letter to the editor [155], commenting Patel et al. [152] high-dose platelet study, the same group explain in detail their rationale, remarking that although injecting very high volumes of PRP (≥15–20 mL) is not common practice—mainly because physicians' habits were shaped by hyaluronic acid protocols (low volume, repeated injections)—scientific elements are justifying such an approach. First, the intra-articular capacity of the knee has been estimated at around 103 mL in healthy joints and 131 mL in osteoarthritic or inflamed knees, supporting the feasibility of higher-volume injections [156,157]. Second, tolerance appears acceptable, with only transient, self-resolving adverse events reported in high-volume protocols. Third, the risk of arthrogenic muscle inhibition has never been described below 20 mL, suggesting that 17 mL PRP is well within the safety margin [158]. Finally, increasing injected volume could enhance diffusion throughout the entire joint cavity and proportionally raise the plasma fraction delivered, providing additional anti-inflammatory mediators such as IGF-1, HGF, IL-1RA, and extracellular vesicles, potentially amplifying the biological effect [159].

These studies directly address the critical issue of dosing in PRP therapy for knee OA, demonstrating that maintaining a high platelet dose (10 billion platelets delivered) confers sustained clinical and chondroprotective benefits over 12 months. The superior outcomes observed support the idea that, beyond concentration, the total platelet dose delivered is a key determinant of clinical efficacy—higher doses produce better and more durable symptom relief and cartilage protection. This work advocates for PRP

standardisation based on absolute platelet count rather than concentration alone, providing a robust evidence base for optimisation of regenerative interventions in knee OA.

5.2 Platelet Dose over Concentration in Tendons and Muscle

The impact of platelet dose in tendinopathy is less clear, as tendinopathies arise from diverse pathophysiological processes—including overuse, degenerative, compressive, neurogenic, and inflammatory mechanisms—which likely influence the variable efficacy of PRP treatments, particularly given the use of both leukocyte-rich and leukocyte-poor formulations. Therefore, optimal platelet dosing may differ among tendinopathy subtypes [150]. Multiple Randomized Controlled Trials (RCTs) and meta-analyses suggest that PRP is superior to corticosteroids and placebo in lateral epicondylitis, with long-term benefits in pain and function [160–164]. Across tendinopathies, clinical outcomes appear dose-dependent, with PRP containing >5–6 billion platelets yielding superior pain relief and functional recovery in rotator cuff, lateral epicondylitis, and gluteal tendinopathy, whereas sub-threshold doses (<5 billion) show little efficacy in patellar tendinopathy, though Achilles tendinopathy and plantar fasciitis may respond even at lower doses [150]

For rotator cuff tendinopathy, PRP injected into the subacromial space or at the tendon–bone interface shows promising results, particularly when guided by ultrasound and delivered repeatedly. In Achilles and patellar tendinopathy, outcomes are more variable and depend heavily on dose, technique, and disease chronicity [165].

A 2024 systematic review and meta-analysis by Ling et al. [166] confirmed that PRP significantly improves pain and function in Achilles tendinopathy compared with placebo and conservative care. However, outcome variability was driven mainly by differences in PRP formulations and delivery protocols, reinforcing the need for standardised dosing and leukocyte profiling.

In their comprehensive chapter, Abelow et al. [167] and Acosta et al. [168] advocated for personalised PRP based on the biological and mechanical context of the tendon, recommending dose calibration, immune profiling, and multimodal delivery (e.g., with needling, shockwave, or loading).

In muscle injuries, optimal dosing is still under investigation [44,150]. Berrigan reported three plantar fasciitis studies with positive outcomes post-injection [150]. Two had platelet doses below 5 billion [169,170], while one fell between 5 and 10 billion [171].

A 2024 systematic review by Vale et al. [172] critically evaluated 11 controlled studies on PRP injections for acute muscle strains in athletes. The analysis found no significant reduction in time to return to sport or prevention of reinjury with PRP treatment compared to controls [172]. However, some evidence suggested potential benefits in pain management during recovery [172]. The review highlighted substantial heterogeneity among study protocols, including variability in PRP preparation, platelet doses, and administration methods, which limits the ability to draw definitive conclusions [172]. The authors emphasised the urgent need for standardised protocols and well-designed clinical trials to assess PRP's efficacy in muscle regeneration better and ensure consistent, reliable treatment outcomes [172].

PRP is not a one-size-fits-all intervention [44]. Its biological and clinical performance depends on total platelet dose, purity, leukocyte profile, and how well it is matched to the clinical target tissue. Future research and clinical practice must move toward precision orthobiologics, abandoning the outdated emphasis on concentration alone.

5.3 PRP Mechanism of Action (MoA): PBMNCs Recruitment over Direct Growth Factors Release

Although platelet dose is traditionally discussed in terms of growth factor availability, within an immune-centric framework, its primary relevance is immunological [6,116].

Higher platelet concentrations amplify the release of key chemokines, most notably CCL2/MCP-1, SDF-1 and PF4, that act as potent recruiters and activators of PBMNCs [115,145,173–175].

To date, no study has quantitatively linked platelet dose, chemokine release, PBMNC recruitment, M2 macrophage polarization and clinical outcomes within a single, disease-specific model. However, the immune-centric framework integrates converging lines of evidence:

(i) Platelet-derived chemokines such as CCL5/RANTES, CXCL4/PF4 and CXCL7/NAP-2 are potent inducers of monocyte recruitment [115,145,173–175]
(ii) PRP and platelet-derived products promote macrophage recruitment and M1→M2 repolarization and enhance tissue repair in preclinical models [176–178]
(iii) Platelet-derived chemokines such as CCL5/RANTES, CXCL4/PF4 and CXCL7/NAP-2 are potent inducers of monocyte recruitment [115,145,173–175]
(iv) Platelet-based products can enrich regulatory, IL–10–producing lymphocytes subsets [179]
(v) Anitua et al. [179] in an extensive review on a total of 27 papers, showed that PRP may play an immune-regulatory role in the macrophage mediated immune response.

This chemotactic signalling is the mechanistic bridge between "platelet dose" and "immune regulation": when platelet numbers exceed a certain threshold, a sufficient gradient is generated to mobilise monocytes from the circulation, guide their trafficking into injured tissues, and promote their transition toward pro-resolutive M2 programs. Consequently, dose optimization in PRP should not be viewed only as a strategy to increase generic trophic factors, but as a requirement to initiate the PBMNC-mediated reparative cascade that underpins effective tissue regeneration. Reframing platelet dose in this way ensures conceptual alignment with the immune-centric paradigm, suggesting that inadequate platelet counts could fail at the level of immune recruitment.

Once recruited, monocytes undergo efferocytosis and polarization, shifting macrophages from an M1 pro-inflammatory phenotype to an M2 reparative phenotype, thereby sustaining resolution of inflammation and tissue repair [6,176–178]. (Fig. 2).

In vitro, Uchiyama et al. [177] demonstrated that PRP directly reprograms macrophages toward an M2 phenotype, even under inflammatory conditions, providing mechanistic proof of its immunomodulatory action.

Data was confirmed in preclinical models, in tendon and cartilage OA models, PRP has been shown to significantly increase local macrophage infiltration and promote M2 polarization, correlating with enhanced healing and pain relief [176,178]. In a tendon healing model, Nishio et al. demonstrated that PRP promotes the recruitment of macrophages, thereby supporting the inflammatory-to-reparative transition essential for tissue regeneration [176]. In a rat model of knee osteoarthritis, Xu et al. showed that PRP recruits macrophages and shifts their polarization from M1 to M2, leading to the resolution of synovial inflammation and relief of pain [178]. Taken together, the evidence from Uchiyama et al. [177], Nishio et al. [176], and Xu et al. [180] indicates that the therapeutic activity of PRP converges on macrophages, promoting their recruitment and polarization from M1 to M2 phenotypes. This shift underlies inflammation resolution, pain relief, and tissue regeneration across *in vitro*, tendon, and osteoarthritis models.

This paradigm redefines PRP as an "immune-centric" biologic, in which the platelet fraction provides chemotactic signals, while monocytes and macrophages are effectors of regeneration through the essential macrophage polarization step.

Consequently, PRP enriched in PBMNC may bridge the gap between conventional platelet-based products and selective PBMNC therapies, aligning clinical practice with the immunological basis of musculoskeletal healing.

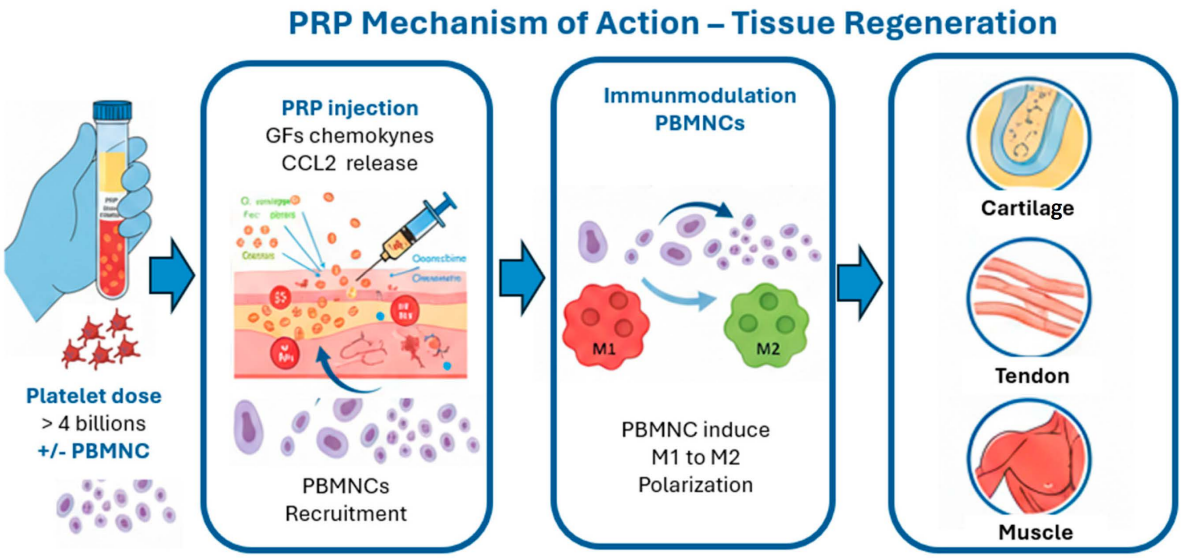

Figure 2: Platelet-rich plasma (PRP) Mechanism of action is primary based on PBMNC recruitment and macrophage polarization.

5.4 PRP Leukocyte Content

Depending on the PRP preparation system used, leukocyte levels can vary significantly, with some formulations being Leukocyte Rich-PRP (LR-PRP) and others Leukocyte Poor PRP (LP-PRP) [44]. Recently, a third, biologically relevant category is emerging: neutrophil-depleted but PRP-PBMNC-enriched [44–46,145,181]. Not all leukocytes exert the same biological functions; while neutrophils and erythrocytes release pro-inflammatory mediators and may exacerbate tissue damage, monocytes and lymphocytes orchestrate immunomodulation, angiogenesis, and tissue repair [44,117,182].

PRP is increasingly understood as an immunobiologic rather than just a regenerative injection. When applied correctly, it recruits and programs immune cells, such as PBMNCs, toward tissue-specific reparative roles [6,116]. This makes PRP a potent immune-recruitment tool, particularly when paired with immune-compatible strategies such as PBMNCs enrichment [44,45].

5.5 PRP Leukocyte Content in Knee OA

Leukocyte concentration is a key variable in PRP formulations for KOA, where the therapeutic goal is to modulate a chronic, low-grade inflammatory environment rather than induce a strong acute inflammatory response. Preclinical and translational work has shown that leukocyte-rich PRP (LR-PRP) increases catabolic cytokines such as IL-1β and TNF-α and up-regulates matrix-degrading enzymes, supporting the concept that an excess of white blood cells may be detrimental in degenerative cartilage disease [183]. Clinical head-to-head double-blind RCTs comparing LR-PRP and LP-PRP in knee OA have reported similar improvements in pain and function at 12 months, with comparable safety profiles, suggesting that leukocytes are not required to achieve the clinical benefit of intra-articular PRP [184,185]. However, network meta-analyses and subgroup analyses indicate that LP-PRP tends to provide greater pain relief than LR-PRP and is associated with fewer inflammatory flares, reinforcing the current preference for leukocyte-poor formulations in this indication [186,187]. This topic will be explored in more detail in the section on PRP enriched with PBMNC, where we will distinguish the pro-inflammatory role of neutrophils from the anti-inflammatory and regenerative functions of monocytes and lymphocytes.

5.6 PRP Leukocyte Content in Tendons

As highlighted in systematic reviews [43] tendinopathy is marked by persistent infiltration of M1 macrophages, leading to ECM degeneration and nociceptive sensitisation. PRP can act as a biological "reset" that restores immune balance and supports matrix reorganisation [164,165,188].

Unlike intra-articular environments, tendon tissue can tolerate—and even benefit from—LR-PRP, especially in early-stage or insertional tendinopathies [145,189–191]. Neutrophils may contribute to initial inflammatory signalling required for tissue turnover, provided their activity is controlled and transient [191]. LR-PRP formulations may be more effective in acute or subacute tendinopathy, enhance local inflammation transiently to stimulate remodelling, and are often combined with needling techniques or dry tenotomy to boost regenerative signalling [191]. In contrast, LP-PRP may be preferred in chronic degenerative tendinopathy with a strong nociceptive component, to reduce flare-ups and avoid unnecessary inflammatory burden [191].

Regarding that tendon regeneration is increasingly recognised as an immune-driven process, *in vitro* studies have shown that macrophage polarization is indispensable for orchestrating extracellular matrix remodelling, angiogenesis, and the resolution of inflammation, with the M1 to M2 switch emerging as a decisive step for tenocyte survival and matrix repair [29]. *In vivo*, murine models of tendon injury have confirmed that regulatory T cells (Tregs) and macrophages cooperate to establish a pro-healing niche; depletion of Tregs impairs neonatal tendon regeneration, whereas their adoptive transfer restores regenerative capacity in adult tendons. [28,124]. Translationally, these concepts are supported by early clinical evidence: in a pilot case series, local injection of autologous PBMNCs into partial Achilles tendon tears promoted rapid pain relief, functional recovery, and imaging evidence of structural repair [35].

Collectively, these findings underscore that tendon healing hinges on a high platelet dose and immune modulation—via PBMNCs, macrophage polarisation, and Treg activity—positioning PRP PBMNC-enriched therapy as an immune-centric strategy, a promising frontier in tendon regenerative therapy.

5.7 PRP Leukocyte Content in Muscle

Skeletal muscle injuries are among the most common conditions in sports medicine, ranging from grade I strains to large myotendinous ruptures [192]. Despite the self-healing nature of muscle tissue, incomplete recovery, fibrosis, and reinjury remain major clinical issues—particularly in high-performance athletes [192].

PRP has emerged as a promising tool to enhance and accelerate myo-regeneration, particularly in the early post-injury phase, when inflammatory and reparative cascades are still active [193].

Controlled-release strategies have emerged to further optimise outcomes and increase the dose in specific environments. Felipone et al. [194] used alginate hydrogels to prolong PRP exposure, reducing fibrosis and improving capillary density. Erfanian et al. [195] used PRP-loaded decellularised ECM scaffolds to enhance myofiber regeneration and innervation. These findings underscore the importance of modulating PRP's spatial and temporal bioactivity.

Acosta et al. [168] proposed tailored PRP formulations based on injury chronicity, patient phenotype, and immune environment. De Sire et al. confirmed PRP's efficacy in pain relief and faster return-to-play in athletes [196]. For acute hamstring injuries, a 2025 systematic review and meta-analysis, including eight papers on 330 patients, was conducted by Liu et al. [197] highlighted that PRP injections, when combined with structured rehabilitation, can shorten return-to-play and may reduce reinjury risk. Notably, the study emphasised that clinical outcomes depend on the timing of administration and the total platelet dose, underscoring the importance of precision dosing strategies in muscle regeneration.

Collectively, these findings position PRP—and particularly PBMNC-enriched PRP—not as a generic biological adjuvant but as a precision immunomodulatory therapy for muscle injuries, where timing, platelet

dose, and immune cell recruitment converge to determine regenerative outcomes and safe, accelerated return to sport.

6 The Next Generation PRP PBMNC Enriched: Not All Leukocytes Are the Same

The recruitment of PBMNC by platelet-derived growth factors released by PRP is a critical step in orchestrating effective tissue regeneration [176–178]. PRP contains a rich mixture of growth factors such as VEGF, PDGF, TGF-β, and IGF-1 that act as potent chemoattractants for immune cells, including PBMNCs [176–178]. Upon recruitment, PBMNCs differentiate into macrophages that regulate the inflammatory response by transitioning from a pro-inflammatory M1 phenotype to a reparative M2 phenotype [176–178]. This polarisation shift is crucial for inflammation resolution, pain relief, and the stimulation of anabolic processes necessary for tissue repair. [176–178].

Recent reviews have explicitly defined "regenerative inflammation" as a framework that integrates the immunological players (especially PBMNCs, macrophage polarization, and timing) with classical regenerative signals [14,31,198]. Caballero-Sánchez et al. [14] describe how a carefully timed infiltration of immune cells, especially monocytes/macrophages, combined with the clearance of debris (efferocytosis), sets the stage for the transition from pro-inflammatory to pro-repair phases in tissue regeneration—including skeletal muscle and cartilage systems.

Martins et al. [117] elucidate this mechanism, emphasising the crucial role of PBMNC present in buffy-coat, in modulating macrophage polarization

- The buffy-coat is a layer of leukocytes and platelets that forms at the top of a red cell sample after centrifugation of peripheral blood in any anticoagulated tube or container.
- Buffy-coat contains a rich mixture of progenitor cells, leukocytes including granulocytes, PBMNCs, platelets, cytokines, and growth factors critical for tissue regeneration.
- PBMNCs, especially monocytes within the buffy coat, play a pivotal role in modulating inflammation and promoting tissue repair via macrophage polarization.
- Macrophage polarization is the switch between pro-inflammatory M1 and anti-inflammatory/pro-regenerative M2 phenotypes; effective tissue regeneration depends on a timely transition to the M2 state.
- Cytokines such as IL-10, TGF-β, and monocyte colony-stimulating factor from buffy-coat promote M2 polarization, thus enhancing tissue repair and resolving inflammation.
- Pro-inflammatory cytokines like TNF-α and IFN-γ stimulate M1 macrophages, which are required initially for pathogen clearance but can impair regeneration if unchecked.
- Signalling pathways (NF-κB for M1; JAK/STAT, PI3K/Akt/mTOR for M2) are modulated by buffy-coat components to regulate macrophage phenotype and inflammation balance.
- Regenerative inflammation mediated by PBMNCs and macrophage polarization fosters an environment conducive to musculoskeletal tissue repair.
- The balance of leukocytes in PRP affects macrophage polarization: leukocyte-rich PRP favours M1, leukocyte-poor PRP favours M2 phenotype.
- Therapeutic strategies leveraging PBMNCs from the buffy-coat aim to optimise macrophage polarization to accelerate tissue regeneration in diseases like osteoarthritis.

These insights reveal that PBMNC-driven macrophage polarisation and PRP-PBMNC-enriched therapies are promising therapeutic targets to enhance regenerative outcomes in clinical applications.

Preclinical evidence has consistently shown that the combination of PBMNCs and PRP exerts synergistic effects on musculoskeletal repair [117]. Platelet chemokines released by PRP can create a powerful

chemotactic gradient (CCL2, CCL5, CXCL12), attracting monocytes and lymphocytes to the damaged tissue: PRP therefore functions not only as a source of growth factors, but primarily as a biological recruiter and immune activator [116,117]. Similarly, the clinical activity of PRP is now understood to rely less on the transient burst of growth factors—whose half-life is measured in minutes—and more on the complex cargo of bioactive signals (including extracellular vesicles, mRNA, and miRNA) that orchestrate immune cell recruitment and activation [6,14,116,117].

This mechanism is relevant across muscle, tendon, and cartilage tissues, forming the basis for the therapeutic efficacy observed with PRP and PBMNC-based regenerative therapies. In this view, PRP acts primarily as a biological recruiter, creating the chemotactic and trophic gradient necessary for monocyte infiltration and polarization.

An *in vitro* study by Yoshida and Murray [129] demonstrated the synergistic effects of PBMNCs and PRP on ligament fibroblasts, with implications for tendon and ligament regeneration. Using a three-dimensional collagen scaffold that simulates the native tendon environment, human anterior cruciate ligament fibroblasts were cultured and treated with PBMNCs, PRP, or their combination over 14 days. The results showed that PBMNCs significantly boost the anabolic effects of PRP by increasing collagen gene expression, protein synthesis, and cell proliferation. The combination enhanced considerably the expression of type I and III procollagen genes, collagen protein production, and fibroblast proliferation compared to either treatment alone. The mechanism involved increased PBMNC-derived interleukin-6 expression stimulated by platelet factors, which promoted anabolic fibroblast activity. Without platelet factors, PBMNC alone did not trigger similar effects, underscoring the critical interplay between immune cells and platelet-derived growth factors in tissue repair. These findings support a promising combined therapeutic approach using PBMNC and PRP for the regeneration of musculoskeletal tissues.

In an ovine tendon injury model, Bronzini et al. [199] compared PB-MSCs expanded from peripheral blood with PRP, demonstrating that while both improve tendon healing, PRP predominantly promoted early inflammation resolution and angiogenesis. In contrast, PB-MSCs supported superior long-term collagen organization and remodeling.

This study by Abdine et al. [107] evaluated the effects of intra-articular injections of PBMNC versus PRP on collagen fibre restoration in the articular cartilage of a rat model of knee osteoarthritis. Thirty-nine adult male albino rats were divided into four groups: control, osteoarthritis model, PBMNC-treated, and PRP-treated groups [107]. Mallory trichrome staining and quantitative morphometric analysis showed a significant, homogeneous decrease in collagen content across all cartilage zones in osteoarthritis rats. PBMNC treatment resulted in a moderate but significant increase in collagen content, primarily in the non-calcified cartilage zone, restoring collagen fibres to near-normal levels [107]. In contrast, PRP treatment caused only a mild increase, with disorganized cellular patterns and reduced collagen compared to controls. The findings suggest that locally injected PBMNCs have a superior regenerative effect on restoring collagen content and cartilage structural integrity compared to PRP in this osteoarthritis model, likely through mechanisms such as angiogenesis, macrophage polarisation, and paracrine stimulation of chondrocytes. This highlights the therapeutic potential of PBMNCs as an autologous cell-based approach for cartilage repair in osteoarthritis or as a PRP-PBMNC-enriched treatment.

Muscle repair involves destruction and inflammation (neutrophils, M1 macrophages), proliferation (satellite cell activation, M2 macrophages), and remodelling (angiogenesis, matrix reorganisation, innervation) [200]. PRP acts on multiple fronts by reducing inflammation via IL-10, IL-1RA, and HGF; promoting myoblast proliferation through IGF-1 and PDGF; enhancing angiogenesis via VEGF; and limiting fibrosis by modulating TGF-β1 signalling and MMP/TIMP balance [192,201].

These effects are most potent when PRP is delivered within 48–72 h post-injury, aligning with the peak of satellite cell activation and M1-to-M2 macrophage transition [194,195].

Incorporation of PBMNCs into PRP enhances these effects by providing a source of monocytes that can differentiate into M2 macrophages and secrete NAMPT [136]. This immunological synergy accelerates regeneration and improves functional outcomes. Human studies show that PRP reduces time to return to sport (RTS) by 15–30% in grade II muscle injuries, lowers reinjury rates when used in structured rehab protocols, and is well tolerated with minimal post-injection flare, especially in neutrophil-depleted formulations [202,203]. PRP-PBMNC-enriched may harness this immune choreography in muscle injury and degeneration [141,142].

6.1 PRP PBMNC Enriched in OA and Degenerated Intervertebral Discs

Saita et al. [46] conducted a retrospective cohort analysis involving 517 patients with KOA treated with three intra-articular injections of PRP-PBMNC, prepared using a single centrifugation method, and showed a significant improvement in patient-reported outcomes (pain and function) at 6 and 12 months post-treatment, with an overall responder rate of 62.1%. Treatment efficacy was higher in patients with mild to moderate KOA (KL grades 2 and 3) compared to severe KOA (KL4), where poor mechanical alignment (femorotibial angle >190°) correlated with diminished response. This PRP preparation is classified as P2-Bβ in DEPA classification, characterised by neutrophil depletion but rich in PBMNCs, suggesting that the quality and cellular composition of PRP, including PBMNC enrichment and low neutrophil content, may play a more critical role.

Our group conducted a retrospective study [45] on 212 patients with knee osteoarthritis (KL grades 1–3) treated with three intra-articular injections of a high-dose neutrophil-depleted PRP preparation, characterised by a high platelet dose (~4 billion platelets per injection) and PBMNC enrichment. Clinical evaluation at 3, 6, and 12 months showed significant improvements in pain (VAS) and function (WOMAC), with responder rates around 70% at all time points, peaking at 6 months [45]. These results closely align with the findings of Saita et al. [46], who used an identical PRP formulation, likewise neutrophil-depleted and PB MNC-rich, reporting similar responder rates in a larger cohort including KL4 patients. The parallel outcomes between these two independent cohorts underline the reproducibility and reliability of this neutrophil-depleted, PBMNC-enriched PRP type for symptomatic early-to-moderate knee osteoarthritis. The absence of serious adverse events in both cohorts further supports the safety of this PRP formulation. Taken together, the overlapping evidence suggests this neutrophil-poor, PBMNC-rich PRP represents an optimised orthobiologic for knee osteoarthritis, warranting further prospective trials and standardisation efforts.

Recent clinical and preclinical evidence emphasises the critical role of PBMNCs in managing KOA. Chuang et al. [34] demonstrated that an intra-articular injection of autologous PBMNCs induces a shift towards anti-inflammatory M2 macrophage polarization, resulting in potent immunomodulatory and regenerative effects comparable to bone marrow mesenchymal stem cells *in vitro* and *in vivo* [34]. Their open-label pilot study with 20 patients (KL II-IV) showed significant and sustained improvement in pain and knee function over 24 months, with excellent safety [34]. Similarly, an observational study by Chiaramonte et al. [37] in 46 moderate KOA patients compared PBMNC injection with hyaluronic acid (HA) and combined PRP-HA therapy were compared, demonstrating comparable pain relief and functional improvement across therapies. However, PBMNCs and PRP-HA achieved superior knee range of motion and functional scores relative to HA alone.

These findings underscore the multifaceted biological effects of PBMNCs, including the secretion of anti-inflammatory cytokines, macrophage polarisation, and facilitation of tissue remodelling, which

contribute to enhanced symptomatic relief and functional recovery in KOA. The enrichment of PBMNCs offers a promising orthobiologic approach that blends immune modulation and regeneration, potentially surpassing conventional treatments by addressing both inflammation and tissue repair mechanisms crucial for early-to-moderate KOA management.

Moreover, a randomised clinical trial evaluated intradiscal injection of PBMNC versus LP-PRP in a particular critical setting, the highly inflamed and hypoxic environment of degenerated intervertebral discs in patients with discogenic chronic low back pain, was performed [42]. Thirty-six patients were randomly assigned to receive conservative care, PRP, or PBMNCs treatment and were followed for six months. The PRP group received 2 mL of leukocyte-poor PRP, prepared from 10 mL of blood (approximately 2.5 billion platelets). In comparison, the PBMNC group received 2 mL of PBMNCs isolated from 100 mL of blood ($6–9 \times 10^7$ cells, without platelet concentration). At six months, only the PBMNCs group showed significant and sustained improvements in both pain (VAS) and disability (ODI) scores, with all patients achieving greater than 50% improvement, whereas PRP provided no significant benefit over conservative care discs [42]. MRI analysis demonstrated that PBMNCs did not reverse disc degeneration but stabilised disc architecture and reduced bone marrow oedema, consistent with their immunomodulatory activity [42]. Both treatments were safe, but PBMNCs produced a superior and more consistent therapeutic effect compared with LP-PRP [42].

Altogether, the accumulating preclinical and clinical evidence suggests that PBMNCs, particularly when combined with PRP, may offer a novel immune-regenerative strategy that surpasses the limitations of conventional biologics, warranting rigorous randomised trials to confirm their therapeutic potential across musculoskeletal disorders. Overall, these data suggest that the evolution of PRP is toward PBMNC-enriched formulations. The underlying immunological shift is simple but fundamental: leukocytes are not interchangeable. Neutrophils propagate acute inflammation and tissue catabolism, whereas monocytes and lymphocytes orchestrate resolution, M2 polarization, and regenerative signalling. Designing PRP around PBMNC enrichment—and not generic "leukocyte levels"—defines the next generation of immune-centric biologics.

Starting from adequate blood volumes, PRP-PBMNC-enriched approximates the PBMNC therapeutic dose obtained with selective filtration POC (≥120 mL, 200 million PBMNCs), in combination with a high platelet dose, bridging conventional PRP and dedicated PBMNC therapy.

6.2 PRP PBMNC Enriched: A Translational Challenge

A major translational challenge is the standardization of PBMNC-enriched PRP formulations. Different preparation systems (e.g., two-step centrifugation vs. selective filtration) can yield 2–5-fold differences in PBMNC counts and neutrophil contamination, making cross-study comparison and dose–response analysis extremely difficult.

To move toward an immune-centric standard, future studies should:

(i) Systematically report the quantitative cellular composition of the injectate (platelet count, PBMNC concentration, neutrophil percentage),
(ii) Converge on a set of minimal technical parameters defining "neutrophil-depleted, PBMNC-enriched PRP" (e.g., specified centrifugation ranges and/or filter membrane characteristics and performance),
(iii) Explore a composite "PBMNC concentration/platelet dose" index as a candidate quality descriptor for immune-regenerative formulations.

At present, such an index remains a research proposal, but it may become crucial for harmonising protocols and linking product characteristics to clinical outcomes in future trials.

Although PBMNC therapy is supported by robust evidence in CLTI and diabetic foot, data in musculoskeletal indications such as rotator cuff tears, meniscal lesions, and knee osteoarthritis are still limited to small cohorts (typically 20–50 patients) and relatively short follow-up (≤24 months).

These studies primarily provide feasibility and safety signals and cannot definitively address long-term comparative efficacy. Future research should therefore prioritise large, multicentre randomized controlled trials directly comparing PBMNCs with PRP, MSC-based products, and standard of care, with follow-up of at least 5 years.

Such trials should integrate validated clinical endpoints, imaging-based structural outcomes, and dedicated safety monitoring, including the risks of intra-articular fibrosis, ectopic tissue formation, and the need for re-intervention.

Evidence in elderly populations: Unlike MSC or adipose-derived preparations, PBMNC therapy has been primarily developed and validated in elderly, fragile CLTI patients. Across published cohorts, including our meta-analysis, the mean age consistently ranges between 71 and 77 years, with high safety and sustained efficacy (improved TcPO2, reduced major amputation, increased healing [33]. These findings indicate that advanced age does not diminish PBMNC therapeutic activity.

However, similar subgroup analyses are currently unavailable for musculoskeletal indications, where the entire field (PRP, MSCs, PBMNC) still lacks age-stratified data. Future trials should include predefined age-based analyses, although at present there is no evidence-based requirement for dose modification in older individuals.

Evidence regarding the effect of obesity on PBMNC-based therapies is currently limited, but available data from related biologics suggest that obesity is unlikely to reduce their efficacy.

A recent secondary analysis of a randomized controlled trial comparing PRP with microfragmented adipose tissue (MFAT) for knee osteoarthritis showed that BMI negatively affected outcomes only in the MFAT group, not in the PRP group [204]. This supports the concept that obesity impairs adipose-derived stromal cell function, while blood-derived immune-regenerative products remain largely unaffected. Although no dedicated analyses exist for PBMNC or PRP–PBMNC formulations, there is no biological rationale to expect reduced activity in obese patients: circulating monocytes, lymphocytes, and platelet-derived mediators retain their immune-modulatory and pro-resolutive functions independently of adipose tissue dysfunction. Given the minimally invasive nature of blood harvesting compared with adipose or bone marrow procedures, PBMNC-based therapies may even represent a practical advantage in obese or metabolically fragile patients.

Beyond formulation standardization, two additional translational aspects deserve consideration. First, current point-of-care systems show variability in PBMNC recovery and leukocyte profiles, reflecting technical differences among centrifugation and filtration devices. While these distinctions do not alter the mechanistic rationale discussed in this review, future technological refinements—including improved separation fidelity or emerging microfluidic approaches—may enhance product consistency and reproducibility.

Second, formal cost-effectiveness analyses for PBMNC or PBMNC-enriched PRP are not yet available. Their minimally invasive nature and outpatient applicability suggest favourable resource utilisation compared with adipose or bone-marrow–derived procedures, but dedicated health-economic evaluations and real-world studies will be needed to quantify long-term value relative to surgical alternatives.

6.3 Repositioning Orthobiologics toward an Immune-Centric Model: Comparative Effectiveness of PRP, MSC-Derived Products, and PBMNC-Enriched Therapies

A clear differentiation among orthobiologic injectables emerges when comparing PRP, minimally manipulated POC MSC-derived products (bone marrow aspirate concentrate (BMAC), microfragmented adipose tissue (MFAT) or SVF mechanically prepared (SVF), and PBMNC-based formulations (PBMNC and PRP enriched in PBMNC)

To provide a concise, clinically oriented synthesis of these approaches, we summarised their mechanisms, evidence levels, strengths and limitations in a comparative table (Table 4).

The most comprehensive and up-to-date clinical recommendations come from the ESSKA–ORBIT Consensus Statements [20,205,206]. In the 2024 PRP consensus, ESSKA recommends PRP as an appropriate injectable option for knee osteoarthritis (KL I–III) after failure of conservative therapy, supported by multiple RCTs and meta-analyses [205,206].

Conversely, the 2025 ESSKA consensus on cell-based therapies concludes that BMAC, MFAT, and other minimally manipulated MSC-containing preparations should not be used as first-line injectable treatments, citing insufficient evidence, lack of superiority over PRP, high biological variability, and practical/technical limitations [20].

These guideline positions are fully consistent with the available randomized controlled trials. Anz et al. demonstrated that PRP and BMAC yield equivalent outcomes at 1 year in symptomatic knee OA, with no added benefit from bone marrow aspiration [207]. Similar findings were reported by Dulic et al., who showed that PRP was comparable or superior to BMAC and hyaluronic acid at 12 months [208].

For adipose-derived therapies, Zaffagnini et al. found that MFAT was not superior to PRP at 2-year follow-up [71]. Likewise, Baria et al. observed equivalence between MFAT and PRP at 12 months, while uniquely showing that high BMI negatively affected outcomes only in the MFAT group, suggesting metabolic fragility of adipose-derived products compared with PRP [70]. PRP combined with hyaluronic acid also performed comparably to MFAT in the prospective randomized trial by Gobbi et al. [209].

At a higher evidence level, a systematic review and meta-analysis by Veronesi et al. concluded that PRP provides more consistent and predictable clinical improvements than adipose-derived minimally manipulated products and carries a more favorable safety and feasibility profile [210]. Additionally, a 2024 network meta-analysis by Jawanda et al., including 48 studies comprising a total of 9338 knees, showed that PRP, BMAC, and hyaluronic acid all outperform corticosteroids at ≥6 months, with PRP exhibiting the most reproducible improvements in pain and function across injectable biologics, measured by Surface under the cumulative ranking curves (SUCRAs) [211].

Taken together, these findings position PRP and emerging PBMNC-enriched PRP formulations as the most evidence-supported and mechanistically coherent orthobiologic options in an immune-centric paradigm. In contrast, minimally manipulated POC MSC-containing products have not demonstrated clinical superiority and are not recommended as first-line injectable therapies in major consensus guidelines.

Table 4: Comparative overview of PRP, MSC-derived products, PBMNC therapy, and PBMNC-enriched PRP in musculoskeletal regeneration.

Therapy	Mechanism of Action	Evidence Level (Cartilage/Tendon/Muscle/Bone)	Guideline Position (ESSKA 2024–2025 & Consensus Statements)	Strengths	Limitations/Gaps
PRP	Platelet-derived chemokines (CCL2, CXCL12) recruit PBMNCs; growth factors modulate inflammation and matrix turnover; promote M1→M2 shift indirectly; improve joint homeostasis.	**Cartilage/OA:** Moderate–High (multiple RCTs). **Tendon:** Moderate (lateral epicondylitis strong, rotator cuff mixed). **Muscle:** Low–Moderate. **Bone:** Moderate (adjunctive).	**ESSKA–ORBIT 2024:** Recommended for Knee OA (Grade A–B). First-line injectable *after conservative failure.* Advised *on* BMAC/MFAT.	Strong RCT support; reproducible clinical improvements; low cost; safe; minimally invasive; metabolic resilience (effective across BMI groups).	High variability between systems; incomplete standardization; variable leukocyte content; need to report platelet dose and PBMNC composition; no single optimal protocol.
MSC-Based Therapies (BMAC, MFAT, SVF)	Paracrine signaling; indirect immunomodulation mainly via apoptosis → efferocytosis → M2 polarization; very low MSC content (0.02–2%); dependent on tissue source health.	**Cartilage:** Low–Moderate (heterogeneous small RCTs; no superiority over PRP). **Tendon:** Low (small case series). **Muscle:** Low (negative in inflamed muscle; fibrosis risk). **Bone:** Moderate (adjunctive).	**ESSKA–ORBIT 2025:** *Not recommended* as first-line injectable therapy for knee OA. No evidence of superiority over PRP. Use only in research or highly selected cases.	Conceptually appealing; one-step harvest; theoretical multipotency; widely available systems.	Weak clinical evidence; inconsistent outcomes; vulnerable to inflammation; MSC proportion extremely low; regulatory limitations; higher procedural burden; reduced efficacy in obesity (MFAT).
Peripheral Blood Mononuclear Cells (PBMNCs)	Direct immune modulation via monocytes → macrophages (M1→M2); IL-10/TGF-β–mediated pro-resolutive pathways; hypoxia-adapted; angiogenic and reparative signaling.	**Cartilage:** Low–Moderate (observational and early controlled studies). **Tendon:** Very low (1 small case series). **Muscle:** Very low (isolated case data). **Bone:** Moderate (preclinical + ischemic tissue). **CLTI/DFU:** High (multiple RCTs, meta-analyses).	No formal guidelines yet. Recognized strong evidence in CLTI/DFU. Emerging use in MSK with promising but limited clinical data.	Strong mechanistic rationale; excellent safety in elderly/fragile patients; robust perfusion benefits in CLTI; minimally invasive harvest; inflammation-tolerant.	Lack of large RCTs in MSK; no defined minimum effective dose; differences among POC devices; need for standardized quantification.
PRP–PBMNC Enriched Formulations	Combines platelet chemotaxis (recruitment) with PBMNC effector cells; amplifies M1→M2 polarization; enhances IL-10/TGF-β pathways and tissue repair.	**Cartilage:** Low–Moderate (case series and small controlled studies). **Tendon:** Very low. **Muscle:** Very low. **Bone:** Emerging evidence; promising in ischemic tissues.	No guideline position yet. Conceptually aligned with immune-centric strategies; under active clinical development.	Mechanistic synergy: potentially more stable and less sensitive to BMI/metabolic factors; minimally invasive; integrates well with existing PRP protocols.	Insufficient standardization; no dose–response data; large RCTs lacking; variability in PBMNC yields across devices (2–5× differences).

Abbreviations: PRP, Platelet-Rich Plasma; MFAT, Microfragmented Adipose Tissue; ESSKA, European Society of Sports Traumatology, Knee Surgery and Arthroscopy; RCT, Randomized Controlled Trial; DFU, Diabetic Foot Ulcer; MSK, Musculoskeletal; IL, Interleukin; TGF-β, Transforming Growth Factor Beta; M1/M2, classically/alternatively activated macrophages; BMI, Body Mass Index; POC, Point-of-Care.

7 Limitations

The rationale behind the use of PBMNCs and PBMNC-enriched PRP is highly compelling and supported by emerging preclinical and preliminary clinical evidence.

Despite the growing body of evidence supporting PBMNC therapy, the current literature is unevenly distributed across clinical indications. Robust prospective cohorts and meta-analytic data exist for CLTI and diabetic foot, positioning these conditions as the most mature areas of application. In contrast, evidence for tendinopathies and muscle regeneration remains extremely limited, consisting of only a small case series and a single case report, respectively. These isolated reports should be interpreted as preliminary biological signals rather than proof of clinical efficacy. This imbalance represents a significant knowledge gap and underscores the need for adequately powered randomized trials, standardized preparation protocols, and longer-term functional follow-up, specifically in tendon and muscle indications. Recognizing this asymmetry is essential for avoiding overgeneralization and for accurately framing the current state of PBMNC research.

Beyond the clinical evidence gaps, several key mechanistic aspects of the immune-centric model remain insufficiently defined. In the process of PBMNC-driven M1→M2 macrophage polarization, the precise division of labor among different cell subsets (e.g., monocytes, macrophages, regulatory T cells) and the quantitative contribution of specific signaling pathways (such as STAT6-mediated alternative activation vs. other transcriptional programs) have not yet been rigorously dissected *in vivo*. Similarly, while PRP-derived chemokines (CCL2, CXCL12, and others) are likely to govern PBMNC recruitment, the dose–response relationship between platelet content, chemokine release, and cell trafficking may vary substantially across disease-specific inflammatory microenvironments (for example, osteoarthritis versus tendinopathy), and remains largely unexplored. Finally, the potential interaction between MSCs and PBMNCs within the same lesion—whether predominantly competitive or synergistic—and the optimal ratio and timing for combined use have not been formally investigated. These unresolved questions highlight the need for focused mechanistic studies, including systems-immunology approaches, *in vivo* cell tracking, and controlled MSC+PBMNC combination models, to refine and validate the immune-centric paradigm.

A major translational limitation in the field is the insufficient characterization and standardization of PRP and PBMNC point-of-care systems. Each device generates a distinct cellular and biochemical profile, with platelet dose, PBMNC yield, and neutrophil content often differing by 2–5-fold. Yet only a minority of systems have published performance data that allow clinicians to understand the expected biological output. For PRP, choosing an appropriate formulation requires knowing both the platelet dose and the leukocyte composition, but these parameters are inconsistently reported and rarely independently validated. The same applies to PBMNC devices, where recovery efficiency, leukocyte distribution, and reproducibility remain poorly documented. Importantly, no study has defined a minimal effective dose or dose–response curve for PBMNC-based therapies or PBMNC-enriched PRP. These gaps limit comparability between studies and hinder evidence-based protocol optimization. Addressing them will require systematic device characterization, transparent reporting standards, and prospective trials designed to link dose parameters with clinical outcomes.

Despite these limitations, the autologous nature, ease of collection, repeatability of treatment, and favourable safety profile position these therapies among the most promising orthobiologics for tissue regeneration. Further well-designed, randomised controlled trials with larger cohorts and standardised protocols are necessary to confirm efficacy, optimise dosing regimens, and establish robust clinical guidelines for different musculoskeletal conditions.

8 Conclusions

Immune cell recruitment is emerging as an efficient, scalable, and cost-effective strategy to enhance revascularisation and tissue regeneration by leveraging the patient's own immune cells. Numerous challenges in tissue repair are linked to delayed or inadequate immune cell recruitment and impaired angiogenesis, which hinder the essential transition from a proinflammatory to an anti-inflammatory phase during healing. The timely and coordinated arrival of immune cells plays a key role in driving the progression from inflammation towards tissue regeneration, thereby supporting functional recovery and stable tissue remodelling.

Moreover, mounting evidence reveals that the predominant mechanism of action of MSCs in inflamed tissues is not engraftment and differentiation. In hostile microenvironments rich in pro-inflammatory cytokines, MSCs undergo apoptosis or fail to engraft; their ultimate contribution is often indirect, since apoptotic MSCs are phagocytosed by M1 macrophages, which subsequently switch towards an M2 reparative phenotype. This process suggests that the true effectors of tissue repair are not the MSCs themselves, but rather monocytes and macrophages that are recruited and reprogrammed within the lesion site [22,23].

This immune-mediated regeneration, while powerful, prompts a fundamental reconsideration of cell source selection. Unlike stromal MSCs from bone marrow or adipose tissue, PBMNCs provide a heterogeneous immune-stromal repertoire naturally adapted to circulating and inflamed environments.

Harvesting PBMNCs circumvents the pitfalls of dysfunctional MSCs from chronically inflamed tissues, delivering a clinically feasible, autologous regenerative cell population that directly harnesses immune-modulatory pathways through monocyte-macrophage crosstalk. This paradigm challenges classical MSC-centric strategies and spotlights PBMNCs as pivotal effectors in immune-driven regenerative medicine, offering superior therapeutic potential in pathologies complicated by chronic inflammation.

Clinical evidence from CLTI and diabetic foot represents the most explicit demonstration that tissue repair is immune-driven. In this setting, PBMNCs demonstrated a superior clinical outcome compared to bone marrow autologous cell therapy (BMMNCs, BMMSCs), suggesting that PBMNCs are the most promising regenerative therapy.

Increasing evidence suggests that PRP acts primarily as a biological recruiter of PBMNCs, creating the chemotactic and trophic gradient necessary for monocyte infiltration and polarisation. Platelet growth factors and chemokines create a powerful chemotactic gradient for PBMNCs, while PBMNCs induce M2 polarization even in a chronic, inflamed, and hypoxic environment.

From this perspective, the therapeutic convergence of PBMNCs and PRP could represent a powerful biological strategy: PBMNCs directly supply the immunocompetent cells capable of driving resolution of inflammation and tissue remodelling, while PRP provides the molecular "call to arms" that amplifies recruitment, survival, and functional reprogramming of these cells. The result is a synergistic immune-regenerative ecosystem in which innate immune cells are both the targets and the main effectors of healing.

High-dose, neutrophil-depleted PRP enriched with PBMNC can complement this strategy by recruiting and implanting PBMNCs simultaneously into the same tissue, aiming to achieve the best clinical outcome.

The immune system itself emerges as the cornerstone of musculoskeletal regeneration. Therefore, as Zarubova has argued, regeneration can be achieved either by: (1) direct implantation of PBMNCs, (2) PRP-mediated recruitment of endogenous PBMNCs, or (3) an autologous combination that integrates both strategies.

The clinical importance of PBMNC-based therapies lies in their unique combination of feasibility, safety, and mechanistic coherence. Unlike adipose- or marrow-derived products, PBMNCs can be obtained

rapidly and minimally invasively, making them suitable even for elderly, obese, or metabolically fragile patients. Their capacity to modulate inflammation, promote M2 macrophage polarization, and support tissue repair offers a biologically targeted strategy for conditions in which chronic inflammation is a central driver of degeneration. These characteristics, together with their compatibility with point-of-care workflows, underscore the translational potential of PBMNC and PBMNC-enriched PRP as clinically relevant, immune-centric regenerative therapies.

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Abbreviations

ADSCs	Adipose-Derived Stromal Cells
ADSVF	Adipose-Derived Stromal Vascular Fraction
AECs	Amniotic Epithelial Cells
AOFAS	American Orthopaedic Foot and Ankle Society Score
BMAC	Bone Marrow Aspirate Concentrate
BM1	Body Mass index
BMMNCs	Bone Marrow Mononuclear Cells
BMMSCs	Bone Marrow Mesenchymal Stem Cells
BMPs	Bone Morphogenic Proteins
CCL2, CCL5, CXCL12, CXCL4	Monocytes chemotactic signals
$CCR2^{+}/Ly6C^{hi}$	Pro inflammatory Monocytes Subpopulation
CFU-F	Colony-Forming Unit-Fibroblast
CLTI	Critical Limb-Threatening Ischemia
COL2	Type II Collagen
COL2A1	Collagen, type II, alpha 1 chain
$CCR2^{+}/Ly6C^{low}$	Anti-inflammatory Monocytes Subpopulation
DEPA	Dose of Platelets, Efficiency, Purity, Activation (classification for PRP)
ECM	Extracellular Matrix
FAPs	fibro/adipogenic progenitors
GFP	Green Fluorescent Protein
HA	Hyaluronic Acid
IKDS	International Knee Documentation Committee (IKDC) scores
IGF-1	Insulin-like Growth Factor 1
IL-1, IL-6, IL-10	Interleukins 1, 6, 10 (cytokines)
IFN-γ	Interferon gamma
KOA	Knee OA
KOOS	Knee Injury and Osteoarthritis Outcome Score

LP-PRP	Leukocyte-Poor Platelet-Rich Plasma
LR-PRP	Leukocyte-Rich Platelet-Rich Plasma
M1	Pro-inflammatory macrophage phenotype
M2	Reparative/anti-inflammatory macrophage phenotype
MFAT	Micro-fragmented Adipose Tissue
MIBO	Minimum Information for Studies Evaluating Biologics in Orthopedics
MMPs	Metalloproteases
MMP/TIMP	Metalloproteases/Tissue Inhibitors Metalloproteases
MoA	Mechanism of Action
MRI	Magnetic Risonance Imaging
MSCs	Mesenchymal Stem/Stromal Cell
MSC-EV	Mesenchymal Stem Cell Extracellular Vesicles
MSC-CM	Mesenchymal Stem Cell Conditioned Media
NAMPT	nicotinamide phosphoribosyltransferase
OA	Osteoarthritis
ODI	Oswestry Disability Index
OMERACT-OARSI	Outcome Measures in Rheumatology (OMERACT) and the Osteoarthritis Research Society International (OARSI).
PBMNCs	Peripheral Blood Mononuclear Cells
PDGF	Platelet-Derived Growth Factor
POC	Point of Care
PRP	Platelet-Rich Plasma
PRP-PBMNC	PRP rich in PBMNC
RAW264.7	Murine macrophage cell line
RCT	Randomized Controlled Trial
ROS	Reactive Oxygen Species
SOX9	Sex determining Region Y-Box 9,
STAT1	Signal Transducer and Activator of Transcription 1
STAT6	Signal Transducer and Activator of Transcription 6
SVF	Stromal Vascular Fraction
TGF-β	Transforming Growth Factor-beta
TKA	Total Knee Arthroplasty
TNF-α	Tumor Necrosis Factor-alpha
VEGF	Vascular Endothelial Growth Factor
VAS	Visual Analogue Scale
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

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