Autologous Orthobiologics

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62.1 Introduction

The human body has an endogenous system of regeneration through stem cells and progenitor cells, signaling cells, and other cell types, as they are found in almost every type of tissue. It is important to understand the terminology used in relation to tissue regeneration.

62.1.1 Regenerative Medicine

Regenerative medicine treatments using autologous PRP, bone marrow preparations, and adipose stem cells can be safely executed by well-trained physicians at the point of care (POC). The objectives of regenerative medicine applications are to support the body to form new functional tissues to replace degenerative or defective ones and to provide therapeutic treatment for conditions where conventional therapies are inadequate.

62.1.2 Orthobiologics

The term orthobiologics has recently been introduced for the treatment of a variety of musculoskeletal (MSK) disorders with autologous orthobiologic preparations such as PRP, bone marrow preparations, and adipose tissue concentrate. Such autologous products are showing promising results for the regenerative capacity of these heterogeneous biological active cellular cocktails. This chapter is not meant to be exhaustive, but our aims are to shed light on cell mechanisms following interventional procedures with orthobiologics. A solid understanding of employing interventional procedures using orthobiological preparations and their cellular responses to MSK disorders and tissue conditions is mandatory. FDA and other regulatory limitations of such products (local or federal) need to be understood by clinicians who have incorporated such treatments in their practice.

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62.1.3 Regenerative Rehabilitation

The American Physical Therapy Association has defined regenerative rehabilitation as the combination of interventional orthobiological techniques and appropriate rehabilitation protocols that harness the bodies' intrinsic healing mechanisms through movement to augment orthobiologic injections [1].

62.2 Description

The purpose of this chapter is to provide detailed information on three autologous orthobiologic preparations: PRP, bone marrow–derived preparations, and adipose tissue concentrate.

62.2.1 Platelet-Rich Plasma

PRP therapies have been used for various indications for more than 30 years, resulting in considerable interest in the potential of PRP in regenerative medicine. Autologous PRP is the processed liquid fraction of autologous peripheral blood with a platelet concentration above the baseline [2]. Currently, PRP therapies are suitable treatment options with clinical benefits and encouraging patient outcomes reported [3–5]. However, new therapeutic insights and needs have

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challenged the practicality and effectiveness of PRP clinical applications as many different commercially available PRP and PRP-like systems are being used [6]. A profuse variability in final PRP cellular contents has been reported. Not surprising, different PRP devices contribute to distinctive PRP properties and bioformulations, which may explain inconsistencies in patient outcomes.

One of the authors (PE) published in 2006 a review on PRP technology, focusing on platelet function, PRP mode of action, platelet growth factor and effects during the various stages of the healing cascade, and the central role of platelet-derived growth factors in various PRP indications. Notably, in this period, PRP-gel research, was directed only toward the ability of PRP to release several platelet growth factors (PGFs) with their specific functions toward only bone growth and bone healing, during orthopedic and spine surgical procedures [7]. Later in this chapter, we will discuss the roles of individual cells that can be present in PRP preparation along with detailed information on their effects on tissue regenerative processes. Furthermore, recent advances in understanding PRP bioformulations, platelet dosing, the specific roles of particular leukocytes, and the effects of PGF concentrations and cytokines on mesenchymal stem cell (MSC) trophic effects [8] will be described, including the pivotal roles of PRP in targeting different cells and tissue environments following cell signaling and paracrine effects [9]. Likewise, we will discuss PRP mechanisms related to inflammation and angiogenesis in tissue repair and regenerative processes. Lastly, we will review the analgesic effects of PRP [10] and the effect of certain drugs on PRP activity.

62.2.2 Bone Marrow Aspirate/Concentrate

Bone marrow concentrates are commonly used autologous regenerative orthobiological therapies. In 1989, Wientroub et al. were the first to describe bone marrow aspiration procedures performed for musculoskeletal applications as a method to improve osteogenic potential of bone grafts in pediatric patients [11]. To produce a BMC, bone marrow aspirate (BMA) must be extracted via an aspiration device, which is inserted through the bony cortex. Bone marrow stroma is collected in syringes. A specific BMA volume is then processed by centrifugation to create a BMC buffy coat that should include mesenchymal and hematopoietic stem cells, myelopoietic and erythropoietic cells, mature leukocytes, platelets, and some megakaryocytes [12]. Later in this chapter, scientific information is provided on the bone marrow aspirate cellular content, their specific biological functions, and intercellular interactions, as these, among others, contribute to tissue regeneration following clinical regenerative medicine applications. Furthermore, we underline the differences between BMA and BMC specimen, both prepared at point of care from freshly aspirated bone marrow.

62.2.3 Adipose Tissue Concentrate

Another source for autologous mesenchymal stem cells is adipose (fat) grafts. Adipose tissue can be harvested via lipoaspiration of subcutaneous fat from various areas of the body like the abdomen, thighs, flank, and perigluteal region [13]. Later in the chapter, we will discuss recent developments in the three autologous orthobiological preparations regarding dosing, cellular structures, cell membrane receptors, and their effects on the innate and adaptive immunomodulatory actions, angiogenesis, and analgesic effects.

62.3 Platelet-Rich Plasma (PRP)

62.3.1 Description

The most well-known physiological role of platelets is the control of hemorrhage, where they accumulate at tissue injury sites and damaged blood vessels. These events are instigated by the expression of integrins and selectins that stimulate platelet adhesion and aggregation, leading to the formation of the platelet plug. However, during PRP preparation procedures, clothing should be always avoided as this will jeopardize the preparation of an adequate and viable e orthobiological injectate.

PRP can be characterized as a complex composition of autologous multicellular components in a small volume of plasma that is acquired from a fraction of peripheral blood after centrifugation. The essentials of a phlebotomy procedure are summarized in Table 62.1. After centrifugation, according to the different cellular densities (where platelets have the lowest density), PRP and their non-platelet cellular constituents can be retrieved from the concentration device.

Table 62.1 Essentials and considerations of a phlebotomy procedure for PRP preparation

Properly labeling relevant syringes with patient information
Sufficient anticoagulant in blood collection syringe
PPE and aseptic techniques
Harvesting site choice and preparation
Proper blood draw and draw time
Agitation of blood with anticoagulant during collection
Controlled transfer to PRP device
Following instructions for use to prepare PRP vial

62.3.1.1 PRP: Preparation

A clear consensus across treatment indications is nonexistent, making it difficult to compare PRP products with their related therapy outcomes. In the majority of reported cases, platelet concentrate therapies are all grouped under the term "PRP," even for the same clinical indication [14]. Therefore, this lack of a consensus in PRP preparation methods and validation continues to contribute to inconsistent PRP patient outcomes, based on enormous differences in PRP formulation, specimen quality, including platelet dosing. Nonetheless, for some medical fields (e.g., OA and tendinopathies), progress has been made in understanding the variations in the PRP formulations, delivery routes, platelet function, and other PRP constituents influencing tissue repair and tissue regeneration.

There are many methods and devices on the market for PRP preparation and this chapter is not promoting any specific device. In Fig. 62.1 (EmCyte Corporation, Fort Myers, FL, USA), cellular density separation of whole blood follows a two-spin centrifugation procedure using the proprietary device. After the first centrifugation procedure, the whole blood components are separated into two basic layers: the platelet (poor) plasma suspension and the RBC layer. After a second centrifugation step, the needed PRP volume can be extracted for patient application. The magnification in Fig. 62.1 shows at the bottom of the device the organized multicomponent buffy coat layer (indicated by the blue lines), containing high concentrations of platelets, monocytes, and lymphocytes, based on density gradients. In this example, a minimal percentage of neutrophils (< 0.3%) and RBCs (< 0.1%) will be extracted, following a neutrophil-poor PRP preparation protocol (modified from Everts et al. (IJMS) [15]).

62.3.1.2 PRP: Platelet Granules and Their Content

PRP should meet the prerequisites (platelet dosage, minimal RBC contamination, and addition or removal of particular leukocytes) to produce significant clinical outcomes. These PRP qualifications, combined with elucidating the activities of different PGFs, platelet proteins, cytokines, and chemokines, contribute to the understanding of the fundamental tissue repair mechanisms involving mitogenesis, angiogenesis, chemotaxis, and extracellular matrix formation.

In early PRP applications, α-granules were the most cited intra-platelet structures because of the presence of many PGFs [16]. To a lesser degree, coagulation factors and regulators of angiogenesis were referenced with PRP applications. Additional factors included less-famous chemokine and cytokine constituents, such as platelet factor 4 (PF4), pro-platelet basic protein, P-selectin (activator of integrin), and the chemokine RANTES (regulated upon activation of normal T cell, expressed and presumably secreted). The overall functions of these specific platelet granule constituents are to recruit and activate other immune cells or induce endothelial cell inflammation (Fig. 62.2) [17].

The number of potential interactions, both direct and indirect, between platelets and other (receptor) cells is wide-ranging. As a result, numerous inflammatory effects can be induced by PRP when applied in a local, pathological, tissue environment. The dense granule constituents like ADP, serotonin, polyphosphates, histamine, and epinephrine are more implicit as modifiers of platelet activation and thrombus formation [18]. Most importantly, many of these elements have immune cell-modifying effects. Platelet ADP is recognized by dendritic cells (DCs), increasing antigen endocytosis. DCs (antigen-presenting cells) are critical for initiating

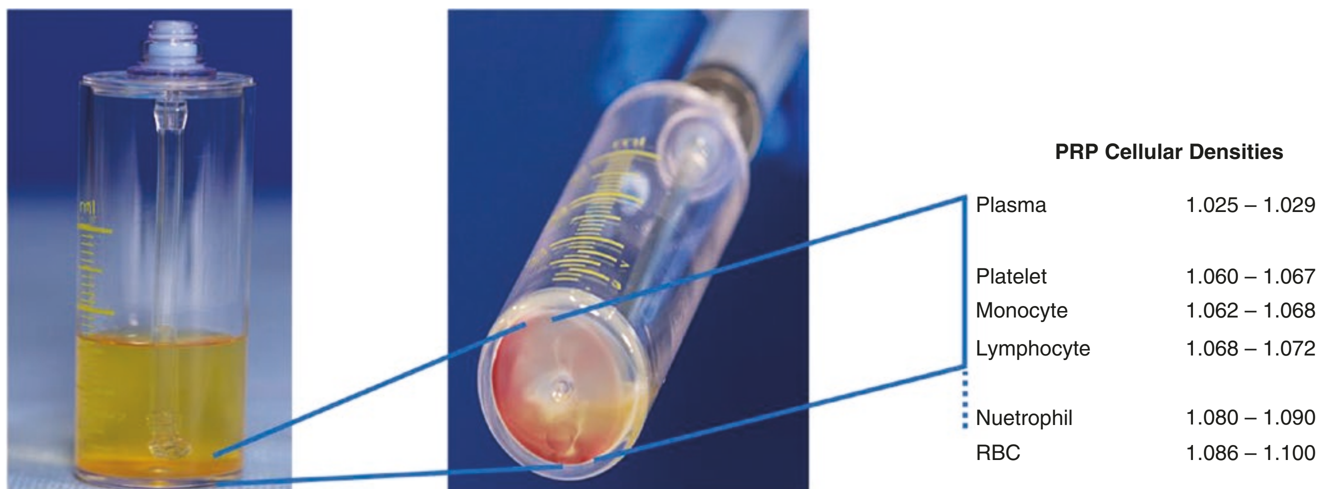

Fig. 62.1 Two-spin PRP preparation procedures result in cellular density gravitational separation. Cells are organized according to their specific densities. (Used with permission from EmCyte Corporation, Fort Myers, FL, USA, PurePRPI® device)

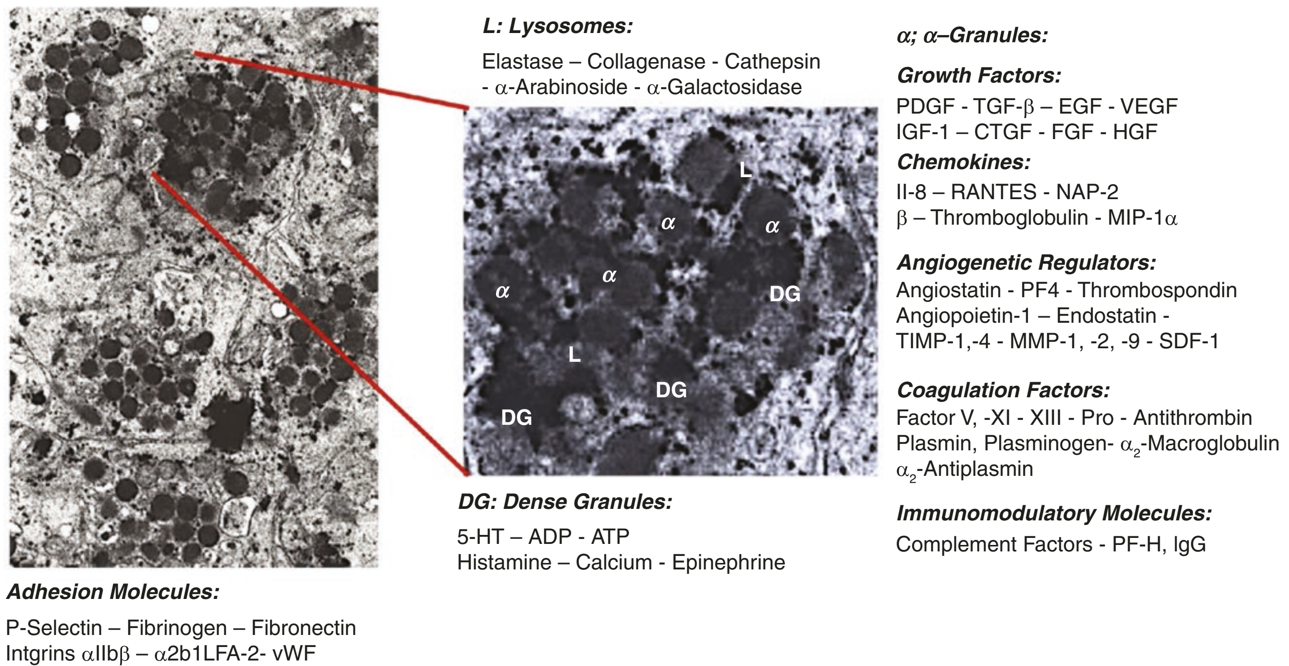

Fig. 62.2 Electron microscopic picture of a cluster of platelets after a PRP preparation showing the various intra-platelet structures at magnification X 10,000. The three platelet cellular constituents α-granules, dense granules (DG), and lysosomes (L) are clearly visible, including some platelet surface adhesion molecules. (Adapted and modified from Everts et al. [15])

T-cell immune responses and govern the protective immune response [18], linking the innate and adaptive immune systems. Moreover, platelet adenosine triphosphate (ATP) signals through the T-cell receptors, which results in an increase in the differentiation of CD4 T-helper cells to pro-inflammatory T helper 17 (Th17) cells [19]. Other platelet-dense granule constituents (e.g., glutamate and serotonin) induce T-cell migration and increase the differentiation of monocyte into DCs, respectively [20]. In PRP, these dense granule–derived immune modifiers are highly enriched and have substantial immune functions.

62.3.1.3 PRP: An Orthobiologic Treatment

PRP preparations have gained increasing popularity with widespread use in diverse medical fields. The underlying scientific rationale for PRP therapy is that an injection of concentrated platelets at sites of injury may initiate tissue repair via the release of many biologically active factors (growth factors, cytokines, lysosomes) and adhesion proteins that are responsible for initiating the hemostatic cascade, synthesis of new connective tissue, and revascularization. PRP is as complex as blood itself and likely more complex than traditional pharmaceutical drugs. PRP products are living biomaterials, and the outcomes of clinical PRP applications are dependent on the intrinsic, versatile, and adaptive characteristics of the patient's blood, including various other cell constituents that may be present in the PRP specimen [21] and the interaction with the recipient local microenvironment, which can be in an acute or chronic state.

PRP concentrates can stimulate the supraphysiological release of growth factors to jump-start healing in chronic injuries and accelerate the acute injury repair process [22]. At all stages of the tissue repair process, a wide variety of growth factors, cytokines, and locally acting regulators contribute to most basic cell functions via endocrine, paracrine, autocrine, and intracrine mechanisms (Table 62.2).

The main advantages of PRP include its safety and the ingenious preparation techniques of current commercial devices to prepare a biologic that can be used in a broad application profile [23]. Most importantly, PRP is an autologous product with no known adverse effects, in contrast to the commonly used corticosteroids and other non-autologous biological products [24, 25]. The enthusiasm to use PRP is often overshadowed as there are no clear regulations regarding the bioformulation and composition of an injectable PRP composition, and PRP compositions vary greatly regarding cellular content and PDGF concentrations [26, 27]. Furthermore, the vital roles of other cellular constituents present in these blood-derived products are partially understood, which was further aggravated by a lack of scientific data, mystical belief, commercial interests, and lack of standardization and classification [14]. In Table 62.3, an overview of the differences between some commercially available PRP and PRP-like devices is displayed, regarding platelet,

Table 62.2 PRP-based growth factors, angiogenetic factors, and platelet cytokines (Partial List)

Growth factors	Function and effects	
	PDGF (AA-BB-AB)	Mitogenic for mesenchymal cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/glial/smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis
	TGF (α – β)	Stimulates undifferentiated mesenchymal cell proliferation; regulates endothelial, fibroblastic, and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; regulates mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis; inhibits macrophage and lymphocyte proliferation
	HGF	Regulates cell growth and motility in epithelial/endothelial cells, supporting epithelial repair and neovascularization during wound healing
	EGF	Proliferation of keratinocytes, fibroblasts, stimulates mitogenesis for endothelial cells
	FGF (a-b)	Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenic for mesenchymal cells, chondrocytes, and osteoblasts
	CTGF	Promotes angiogenesis, cartilage regeneration, fibrosis, and platelet adhesion
	IGF-1	Chemotactic for fibroblasts and stimulates protein synthesis; enhances bone formation by proliferation and differentiation of osteoblasts
	KGF	Regulates epithelial migration and proliferation
Angiogenetic factors	VEGF	Increases angiogenesis and vessel permeability; stimulates mitogenesis for endothelial cells
	IL-8	Pro-angiogenetic to stimulate angiogenesis
	5-HT	Pro-angiogenetic contributions
	Ang-1	Induces angiogenesis stimulating migration and proliferation of endothelial cells; supports and stabilizes blood vessel development via the recruitment of pericyte
	Endo	Endostatin
Cytokines	IL-1	Promotes systemic inflammation
	IL-6	Pro inflammation – anti-inflammation
	PF-4	Calls leucocytes and regulates their activation; has anti-angiogenetic properties
	SDF-1α	Calls CD34+ cells, induces their homing, proliferation, and differentiation into endothelial progenitor cells stimulating angiogenesis; calls mesenchymal stem cells and leukocytes
	TNF	Regulates monocyte migration, fibroblast proliferation, macrophage activation, angiogenesis

Modified from Everts et al. [32]
Abbreviations: *PDGF* platelet-derived growth factors, *TGF* transforming growth factor, *VEGF* vascular endothelial growth factor, *EGF* epidermal growth factor, *FGF* fibroblast growth factor, *CTCG* connective tissue growth factor, *IGF* insulin-like growth factor, *HGF* hepatocyte growth factor, *KGF* keratinocyte growth factor, *5-HT* serotonin, *Ang-1* angiopoietin-1, *Endo* endostatin, *IL-1* interleukin 1, *IL-6* interleukin 6, *IL-8* interleukin 8, *PF4* platelet factor 4, *SDF* stromal cell–derived factor, *TNF* tumor necrosis factor

Table 62.3 Variances in PRP commercial products and specimen differences

	Angel®	GPSIII®	PurePRP-A®	SmartPrep®	Regenkit®-A-PRP
PLT increase from BL	4.8	4.2	6.6	4.9	0.6
Platelets (× 10^6/mL)	856	754	1175	882	107
WBC (× 10^6/mL)	7.1	19.8	10.7	21.4	0.3
Monocyte %	33	15	72	27	0
RBC (× 10^9/mL) /HCT	0.3/2.8	1.1/8.1	0.1/1.1	0.9/7.9	0/0
PRP volume (mL)	3	6	7	7	5
T.D. PLTs (× 10^6/mL)	2568	4524	8225	6174	535

Same donor laboratory study (*N* = 12, only male donors), with average baseline platelet count of 178 × 10^6/mL, unpublished data
Abbreviations: *PLT* platelet, *BL* baseline, *WBC* white blood cell, *RBC* red blood cell, *PRP* platelet-rich plasma, *TD PLT* total deliverable platelets
Angel: Arthrex, Naples, FL, USA; GPS III: Zimmer Biomet, Warsaw, IN, USA; PurePRP: EmCyte Corporation, Fort Myers, FL, USA; SmartPrep: Terumo, Lakewood, CO, USA; Regenkit-A-PRP: Mont-sur-Lausanne, Switzerland

leukocyte cell (WBC) content, red blood cell (RBC) contamination, preparation volumes, and platelet dosing capabilities.

62.3.1.4 PRP Classification

Currently, orthobiological applications classify PRP into three groups: pure platelet-rich fibrin (P-PRF), leukocyte-rich PRP (LR-PRP), and leukocyte-poor PRP (LP-PRP) [28]. Although more specific than a generic PRP product definition, the LR-PRP and LP-PRP categories are significantly lacking any specificity regarding the leukocyte content. Regrettably, there is no consensus on a comprehensive classification system for PRP or any other autologous blood and blood-derived preparations. Ideally, a classification system should focus on the various PRP characteristics, definitions, and appropriate nomenclature that are relevant for therapeutic decision-making to treat patient-specific conditions.

62.3.2 PRP Component: Platelet

62.3.2.1 Platelet Activation and Tissue Repair Mechanisms

After the application of PRP on diseased tissues to initiate repair mechanism, PRP platelets interact with a broad range of cells to induce regenerative tissue remodeling mechanisms, following the classic functions of platelets. Underestimated are platelet functions where they interrelate with a variety of immune cells to regulate the immune responses following tissue injury and inflammation [29, 30]. During traumatic MSK disorders, platelets are among the first cells arriving at the site of vascular lesions and tissue breaches, where they interact with leukocytes, endothelial cells, and resident or circulating cells that are involved in tissue reorganization, following the induction and regulation of hemostasis [31].

After a controlled delivery of non-activated PRP, the platelets will be activated by interacting with platelet tissue factor (factor III), present in subendothelial tissues and leukocytes. Other activation pathways can be activated by the addition of $CaCl_2$ and/or thrombin preparations.

Following platelet activation, the platelet α-, dense, lysosomal, and T granules undergo regulated exocytosis and release their contents into the extracellular environment (Fig. 62.3) [33, 34]. As a result, a platelet plug will develop in the injected microenvironment, as the first step of the healing cascade with the release of signaling molecules that trigger the recruitment and activation of inflammatory cells through a broad range of cell membrane receptors and soluble mediators, which are released upon PRP platelet activation [35].

62.3.2.2 PRP: Critical Platelet Count

PRP treatment protocols have evolved immensely over the past 10 years. Through experimental and clinical research, we now have a better understanding of platelet and other cellular physiology. Systematic reviews, meta-analyses, and randomized controlled trials denote the effectiveness of PRP biological technologies in many medical fields, including orthobiology and sports medicine [36, 37], spinal disorders [38], dermatology [39], cardiac surgery [40], plastic surgery [41], orthopedic surgery [42], and pain management [43].

The therapeutic actions of PRP and other platelet concentrates stem from the release of a multitude of factors involved in tissue repair and regeneration. Following platelet activation, a platelet plug is formed, which acts as a temporary extracellular matrix, allowing cells to proliferate and differentiate [2]. Therefore, it is fair to assume that higher platelet dosages will generate an elevated local concentration of released platelet bioactive factors. However, the correlation between platelet dose, concentration, and the concentration of released platelet bioactive growth factors and agents may not be precise because there are marked differences in baseline platelet counts between individual patients [44], and differences exist between PRP preparation methods [45, 46]. Likewise, several platelet growth factors involved in tissue repair mechanisms reside in the plasma fraction of PRP (e.g., hepatic growth factor and insulin-like growth factor 1). Therefore, higher platelet concentrations do not affect the repair potential of PGF [47].

In in vitro PRP research, study results are quickly obtained because the different parameters can be precisely controlled. Several studies have demonstrated that cells respond to PRP in a dose-dependent manner. Nguyen and Pham [48] showed

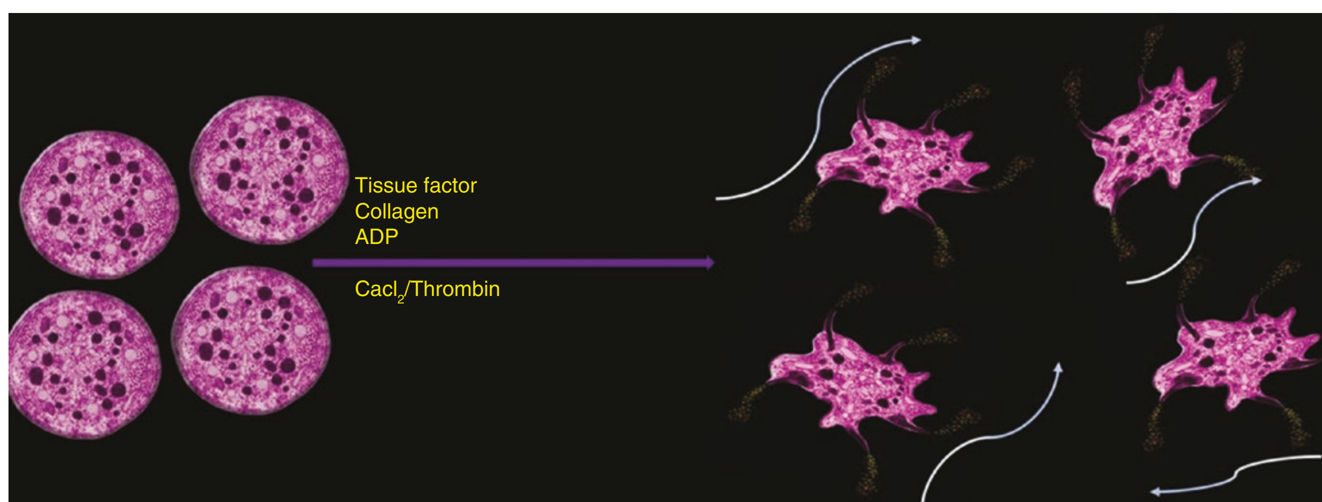

Fig. 62.3 Activated platelets change their shape with the development of pseudopods to promote platelet aggregation. Passive platelet activation is induced by platelet tissue factor and collagen, while active platelet activation can be accomplished by adding $CaCl_2$ and/or thrombin to the PRP specimen. Platelet activation will release the platelet content and all cellular components will invade the tissues

that very high concentrations of PGF are not necessarily advantageous for cell stimulatory processes and may be counterproductive. Some in vitro studies have indicated that high PGF concentrations may have detrimental effects [49]. One reason could be that the quantity of cell membrane receptors is limited. Thus, once the PGF levels are too high compared to the available receptors, they negatively affect cell function [50]. Although in vitro studies have many advantages, they also have some weaknesses.

62.3.2.3 PRP: Platelet Function: In Vitro vs. In Vivo

Due to tissue architecture and cell organization, there is a continuous interplay between many different cell types within any tissue, making it difficult to replicate in vitro in a two-dimensional monoculture setting. Cell density, which can affect cell signaling pathways, is usually less than 1% of the tissue situation. The two-dimensional in vitro culture dish organization precludes cells from being exposed to an extracellular matrix (ECM). Furthermore, typical culturing techniques lead to the accumulation of cellular waste products and continuous nutrient consumption. Thus, in vitro culturing does not resemble any homeostatic conditions, the tissue oxygen supply, or the sudden exchange of media, making it difficult to translate in vitro PRP dosing results into clinical practice. Conflicting results have been published comparing the clinical effects of PRP to in vitro studies for specific cells, tissue types, and platelet concentrations. Graziani et al. [51] found that in vitro, the maximum effect on the proliferation of osteoblasts and fibroblasts was achieved at a PRP platelet concentration that was 2.5-fold times higher than the baseline value. In contrast, clinical data presented by Park and associates [52] indicated that more than a fivefold increase in PRP platelet levels above baseline was required to induce a positive outcome after spinal fusion. Similar contradictory outcomes have been reported between in vitro tendon proliferation data and clinical outcome studies [36, 37]. It is apparent that in vitro and animal methodologies are not ideal study settings for successful translation into clinical practice.

62.3.2.4 PRP: Variation in Preparation

In general, PRP device comparison studies should not support decision-making, as they indicate a large variation in platelet concentrations among the large variety of available PRP devices [14], as demonstrated in an extensive review by Fadadu et al. [6]. In their study, 33 PRP systems and protocols were evaluated. Some of these systems produced final PRP preparations with a platelet count less than that of whole blood. They reported a PRP platelet factor increase as low as 0.52 with a single spin kit [53]. In contrast, the dual-spin device produced the highest platelet concentration (1.6×10^6/μL) [6].

62.3.2.5 PRP: Clinically Required Platelet Concentration

It is important to understand the minimally required platelet concentration to induce an angiogenic response and stimulate cell proliferation and cell migration. Currently, PRP is characterized by its absolute platelet concentration, thereby shifting from the initial definition of PRP consisting of a platelet concentration above baseline values [2] to a minimum platelet concentration of more than 1×10^6/μL or an approximately fivefold increase in platelets from baseline [54]. “Clinical PRP” should contain a critical dose of concentrated platelets to produce beneficial therapeutic effects. The platelets in clinical PRP should stimulate cell proliferation, synthesize mesenchymal and neurotrophic factors, contribute to chemotactic cell migration, and stimulate immunomodulatory activities [55, 56]. Marx was the first to demonstrate the enhancement of bone and soft tissue healing with a minimum platelet count of 1×10^6/μL [2]. These results were confirmed in a transforaminal lumbar fusion study that demonstrated significantly more fusion when the platelet dose was greater than 1.3×10^6 platelets /μL [57]. Moreover, Giusti et al. [58] revealed that a dose of 1.5×10^9 platelets/mL is needed for tissue repair mechanisms to induce a functional angiogenic response through endothelial cell activity. In Giusti’s study, higher concentrations reduced the angiogenic potential of platelets in follicular and perifollicular angiogenesis. Furthermore, earlier data indicate that the PRP dose also affects the magnitude of the therapy outcome [59]. Therefore, to significantly induce an angiogenic response and stimulate cell proliferation and cell migration, clinical PRP should contain at least 7.5×10^9 deliverable platelets in a 5-mL PRP treatment vial.

62.3.2.6 PRP: Dose Dependency

Apart from dose dependency, the effects of PRP on cell activity appear to be highly time-dependent. Soffer et al. [60] indicated that short-term exposure to human platelet lysate stimulates bone cell proliferation and chemotaxis. In contrast, long-term PRP exposure results in decreased levels of alkaline phosphatase and mineral formation. In a prospective, double-blinded, randomized controlled study from Bansal and associates, the effect of platelet dosing was unambiguously presented in patients with knee OA [61] This is the first study addressing the effect of leukocyte-poor PRP-platelet dosing. In their study, a single injection of 10 billion platelets, in a volume of 8 ml, improved functional and pain outcomes and preserved the articular cartilage for 12 months. Furthermore, decreased inflammatory markers were noted. From this study, one might conclude a sustained therapeutic effect with this high dose of platelets. Further studies need to elaborate whether higher doses are more beneficial or less effective.

62.3.3 PRP Component: Leukocytes

62.3.3.1 PRP: Leukocyte Fraction

The presence of leukocytes in PRP treatment is variable and dependent on the PRP preparation device. The presence of leukocytes in PRP preparations is dependent on the manufacturer's instruction and preparation protocol. The so-called plasma-based PRP devices do not contain any leukocytes. In buffy coat layer of PRP preparations, leukocytes are significantly concentrated [62], except for the eosinophils and basophils, as their cell membranes are too fragile to withstand the centrifugal processing forces.

Systematic reviews identified LP-PRP as the preferred PRP formulation to achieve effective treatment outcomes for joint OA [63]. However, Lana et al. [64] disagreed with this theory, suggesting that particular leukocytes play an important role in the inflammatory process preceding tissue regeneration due to their release of both pro- and anti-inflammatory molecules. They found that the combination of neutrophils and activated platelets could have a more positive than detrimental effect on tissue repair. They also indicated that the plasticity of monocytes is important for the non-inflammatory and reparative roles in tissue repair [64].

It is generally accepted that leukocytes greatly impact the intrinsic biology of chronic tissue lesions due to their immune and host-defense mechanisms. Much has been debated about the presence or absence of leukocytes and their contributions to different sub-PRP products, as reflected in Table 62.4 [64, 65]. In a recent review, six randomized controlled trials (level 1 evidence) and three prospective comparative studies (level 2 evidence) with a total of 1055 patients showed that LR-PRP and LP-PRP had similar safety profiles [66]. The authors concluded that the adverse reactions from PRP might not be directly related to the leukocyte concentration. In another study, LR-PRP did not modify systemic or local levels of the pro-inflammatory interleukins (IL-1β, IL-6, IL-8, and IL-17) in OA knees [67]. Those results support the idea that the in vivo role of leukocytes in the bioactivity of PRP might come from the crosstalk between the platelets and leukocytes. This interaction could promote the biosynthesis of other factors (e.g., lipoxins) that counteract or facilitate the resolution of inflammation [68].

After the initial release of inflammatory molecules (arachidonic acid, leukotrienes, and prostaglandins), lipoxin A4 is released from activated platelets to prevent neutrophil activation [64]. It is in this milieu that switches the MΦ phenotypes, from MΦ1 to MΦ2 [68]. Moreover, there has been accumulating evidence indicating that circulating monocytes can differentiate into a variety of non-phagocytic cell types due to their multipotential nature [69].

Table 62.4 PRP and sub-PRP classifications

Platelet-rich plasma (PRP)
Pure-PRP (P-PRP)
Leukocyte-rich PRP (LR-PRP)
Leukocyte-poor PRP (LP-PRP)
Platelet-rich fibrin (PRF)
Platelet-rich fibrin matrix (PRFM)
Preparation rich in growth factors (PRGF)

The type of PRP can influence MSC cultures. LR-PRP can induce significantly higher bone marrow–derived MSC (BM-MSC) proliferation than pure PRP or PPP samples, with faster release and better biological activity of PGFs [70].

62.3.3.2 PRP: Function of Specific Leukocyte

Leukocytes greatly influence the intrinsic biology of acute and chronic tissue conditions because of their immune and host-defense mechanisms. Therefore, the presence of specific leukocytes in PRP preparations can cause significant cellular and tissue effects. More specifically, different PRP buffy coat systems utilize different preparation protocols, thereby producing different neutrophil, lymphocyte, and monocyte cell ratios in PRP [71]. Further research is needed to develop a consensus regarding the role and magnitude of leukocytes in PRP bioformulations to treat certain pathologies and conditions adequately and safely.

- *Neutrophils*

Neutrophils are essential leukocytes in numerous healing pathways that create dense barriers against invading pathogens [72] in conjunction with antimicrobial proteins present in platelets [73]. The presence of neutrophils in PRP preparations is mostly based on the treatment objectives. Exacerbated tissue inflammatory levels can be necessary in chronic wound care PRP biological treatments [32] or applications directed toward bone growth or healing [74]. The use of a full buffy coat PRP treatment vial is also frequently mentioned in chronic tendinopathy treatments [75, 76]. Importantly, additional neutrophil functions have been uncovered in several models, emphasizing their roles in angiogenesis and tissue restoration [77]. However, neutrophils can also cause harmful effects and, thus, are not indicated for some applications. Zhou and Wang demonstrated that the use of PRP rich in neutrophils could result in a higher collagen type III to collagen type I ratio, adding to fibrosis and decreased tendon strength [78]. Other neutrophil-mediated deleterious properties are the release of inflammatory cytokines and metalloproteinases (MMPs) that promote pro-inflammatory and catabolic effects when applied to tissues [79].

- *Lymphocytes*

In the buffy coat of PRP preparations, mononuclear T and B lymphocytes are more concentrated than any other

leukocytes. They are critically involved in cell-mediated cytotoxic adaptive immunity. Lymphocytes can elicit a cell response to fight infection and adapt to intruders [80]. Furthermore, T lymphocyte–derived cytokines (interferon-γ [IFN-γ] and interleukin-4 [IL-4]) strengthen macrophage polarization [81]. Weirather et al. demonstrated that regular T lymphocytes indirectly contribute to tissue healing in a mouse model by modulating monocyte and macrophage differentiation [82].

- *Monocytes and Macrophages*

Depending on the PRP preparation devices used, monocytes may be prominent or absent in prepared PRP. Unfortunately, their manifestation and regenerative capabilities are rarely discussed in the literature. Therefore, little attention is given to monocytes in preparation methods or final formulations. In the orthobiological literature, leukocyte differentiation is rarely addressed and the reporting of PRP preparation protocols has been highly inconsistent. Furthermore, most published studies do not present the PRP preparation methods needed for protocol reproducibility, even though PRP biological preparations containing specific leukocytes can significantly contribute to pro-inflammation, immune modulation, tissue repair, and regeneration. However, monocytes and macrophages play key roles in immunomodulatory processes and tissue repair mechanisms [83].

Monocyte populations are heterogeneous and originate from progenitor cells in the bone marrow via hematopoietic stem cell pathways and traffic via the bloodstream to peripheral tissues depending on the microenvironmental stimuli. During homeostasis and inflammation, circulating monocytes leave the bloodstream and are recruited to injured or degenerated tissues. They can act either as effector cells or as progenitors of macrophages (MΦs). Monocytes, macrophages, and dendritic cells represent the mononuclear phagocyte system (MPS) [84]. A typical feature of the MPS is the plasticity in their gene expression patterns and functional overlap between these cell types. In degenerated tissues, resident macrophages, local-acting growth factors, pro-inflammatory cytokines, apoptotic or necrotic cells, and microbial products initiate the differentiation of monocytes into MPS cell populations [85]. Hypothetically, when C-PRP containing high yields of monocytes is injected in a diseased local microenvironment, monocytes most likely differentiate into MΦs to provoke major cellular changes.

During the monocyte-to-MΦ transition, particular MΦ phenotypes are produced [83]. MΦ phenotype 1 (MΦ1) is characterized by inflammatory cytokine secretion and the production of both VEGF and FGF. The MΦ2 phenotype consists of anti-inflammatory cells, producing mainly extracellular matrix components and angiogenic factors [84]. From these data, it is reasonable to assume that C-PRP preparations containing a high concentration of monocytes and MΦs are likely to contribute to better tissue repair because of their anti-inflammatory tissue repair and cell signaling capabilities.

62.3.4 PRP Component: RBC

The role of RBCs in tissue regeneration has never been established. RBCs are responsible for transporting oxygen to tissues and removing carbon dioxide from tissues to the lungs [86]. They have no nucleus and are made of protein-bound heme molecules. Iron and heme components inside RBCs facilitate the binding of oxygen and carbon dioxide. Normally, the RBC life cycle is approximately 120 days. They are removed from circulation by macrophages by a process termed RBC senescence. Under conditions of shear forces (e.g., whole blood phlebotomy procedures, immune-mediated processes, oxidative stress, or inadequate PRP concentration protocols), RBCs in the PRP specimens could become damaged. As a consequence, the RBC cell membrane disintegrates and releases toxic hemoglobin (Hb), measured as plasma-free hemoglobin (PFH), hemin, and iron [87]. PFH and its degradation products (heme and iron) collectively lead to detrimental and cytotoxic effects on tissues, causing oxidative stress, loss of nitric oxide, activation of inflammatory pathways, and immunosuppression. These effects ultimately lead to microcirculatory dysfunction, local vasoconstriction with vascular damage, and significant tissue injury.

Most importantly, when PRP containing RBCs is delivered to tissues, it causes a local response called eryptosis, which triggers the release of a potent cytokine, macrophage migration inhibitory factor [88]. This cytokine inhibits the migration of monocytes and macrophages. It exerts profound pro-inflammatory signals to surrounding tissues that inhibit the migration of stem cells and fibroblast proliferation and causes significant local cellular dysfunction. Based on above explanation, limiting RBC contamination in PRP preparations is important. Adequate C-PRP centrifugation and preparation processes typically reduce or even eliminate the presence of RBCs, thereby avoiding the detrimental consequences of hemolysis and eryptosis.

62.3.5 PRP: Immunomodulatory Effects

The body can identify foreign bodies and injured tissues in acute or chronic conditions to initiate the wound healing cascade and related inflammatory pathways. The innate and adaptive immune systems protect the host from infection, with essential roles for leukocytes overlapping between both systems, as displayed in Fig. 62.4. Specifically, monocytes,

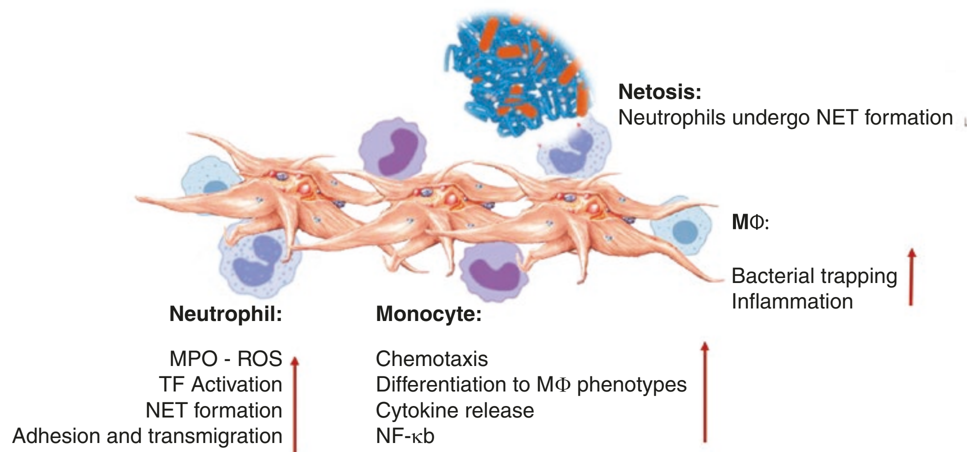

Fig. 62.4 Platelet and leukocyte interactions in innate immunity cell interactions. Platelets interact with neutrophils, monocytes, and ultimately with MΦs, modulating and increasing their effector functions. These platelet-leukocyte interactions result in inflammatory contributions through different mechanisms, including NETosis. Abbreviations: MPO myeloperoxidase, ROS reactive oxygen species, TF tissue factor, NETs neutrophil extracellular traps, NF-κB nuclear factor kappa B, MΦ macrophage

macrophages, neutrophils, and natural killer cells have pivotal roles in the innate system, whereas lymphocytes and their subsets play similar roles in the adaptive immune system [89].

- *Innate Immune System*

The role of the innate immune system is to identify intruding microbes or tissue fragments and stimulate their clearance. Activation of the innate immune system occurs when certain molecular structures, termed *surface-expressed pattern recognition receptors*, bind to *pathogen-associated molecular patterns* and *damage-associated molecular patterns*. Interestingly, platelets also express several immunomodulatory receptor molecules on their surface, such as P-selectin, transmembrane protein CD40 ligand (CD40L), cytokines (e.g., IL-1β), and platelet specific toll-like receptors (TLR), enabling them to interact with various immune cells [90].

Neutrophils, monocytes, and dendritic cells are the most common innate immune cells in the blood. Their recruitment is required for an adequate early-phase immune response. Platelet-leukocyte interactions regulate inflammation, wound healing, and tissue repair when PRP is used in regenerative medicine applications. More specifically, the platelet TLRs stimulate platelet-neutrophil interactions [91], which regulate the so-called leukocyte oxidative burst by modulating the release of reactive oxygen species (ROS) and myeloperoxidase (MPO) from neutrophils [92]. Furthermore, the platelet-neutrophil interaction with neutrophil degranulation results in the formation of neutrophil-extracellular traps (NETs). NETs comprise the neutrophil nucleus and other neutrophil intracellular contents that trap bacteria and kill them by NETosis. The formation of NETs is an essential killing mechanism for neutrophils [93]. As a result of PRP platelet activation, monocytes can migrate to diseased and degenerative tissues where they perform adhesion activities while secreting inflammatory molecules that may alter chemotaxis and modify proteolytic properties [94]. Additionally, platelets can modulate the effector functions of monocytes by inducing the activation of monocyte NF-κb [95], a critical mediator of the inflammatory response and the activation and differentiation of immune cells. Therefore, PRP preparation devices that can yield high concentrations of monocytes from whole blood have the advantage of this mechanism in tissue repair processes after PRP application.

- *Adaptive Immune System*

The adaptive immune system employs antigen-specific receptors and remembers previous pathogen encounters and destroys these pathogens during subsequent encounters with the host. However, these adaptive immune responses are slow to develop. Cognasse et al. [90] showed that platelet components contribute to danger sensing and tissue repair and suggested that the interaction of platelets with leukocytes facilitates the activation of the adaptive immune response.

During adaptive immune responses, platelets promote monocyte and macrophage responses. Thus, platelet granular constituents directly affect adaptive immunity by expressing CD40L [96], a molecule critical to the modulation of adaptive immune responses, as they promote T-cell responses to inflammatory stimuli for robust pro-and anti-inflammatory responses [97]. Moreover, platelets have an abundance of cell surface receptors that can prompt platelet activation,

with the release of numerous inflammatory and bioactive molecules stored within different platelet granules, thus influencing both innate and adaptive immune responses [30].

Following the identification of microbes or tissue damage by the non-specific innate immune system, the specific adaptive immune system takes over. The adaptive system includes B lymphocytes (B cells), which bind antigens, and regular T lymphocytes (Treg), which coordinate the elimination of the pathogens. T cells can be broadly categorized into helper T cells (Th cells) and cytotoxic T cells (Tc cells, also known as T killer cells) [89]. The Th cells are further divided into Th1, Th2, and Th17 cells, with critical functions in inflammation. The Th cells can secrete pro-inflammatory cytokines (e.g., IFN-γ and TNF-β) and several interleukins. They are particularly effective in protecting against intracellular viral and bacterial infections. Th cells stimulate proliferation and differentiation of cells involved in the immunological response. Tc cells are effector cells that eliminate the targeted intracellular and extracellular microbes and cells [98].

Interestingly, the Th2 cells produce IL-4 and influence MΦ polarization, directing MΦs to the regenerative MΦ2 phenotype, while IFN-γ shifts MΦ toward the inflammatory MΦ1phenotype, depending on the dose and timing of the cytokines. Th cells guide MΦ phenotypes to pro-regenerative phenotypes in response to tissue-derived biologics in an IL-4-dependent manner [99]. This mechanism is based on the evidence that Th cells have a pronounced role in controlling both inflammation and tissue repair.

62.3.6 PRP: Nociceptive Effects

Activated platelets release many pro- and anti-inflammatory mediators that are proficient in inducing pain but can also reduce inflammation and pain. Once applied, the typical platelet dynamics of PRP alter the microenvironment prior to tissue repair and regeneration via multiple complex pathways related to anabolic and catabolic processes, cell proliferation, differentiation, and stem cell regulation. These PRP characteristics have led to the implementation of PRP applications in various clinical pathological conditions that are usually associated with chronic pain (e.g., sports injuries, orthopedic pathologies, spinal disorders, and complex chronic wounds), even though the exact mechanisms are not yet fully understood.

In 2008, Everts et al. [100] were the first to report a randomized controlled trial on the analgesic effects of a PRP formulation prepared from autologous buffy coat and activated with autologous thrombin following shoulder surgery. They noticed a significant reduction in visual analog scale scores, the use of opioid-based pain medication, and a more successful postsurgical rehabilitation. Of note, they reflected on the analgesic effects of activated platelets and postulated on the mechanism of platelet-released 5-HT. Briefly, platelets are dormant in freshly prepared PRP. After direct or indirect (tissue factor) platelet activation, platelets change shape and develop pseudopods to promote platelet aggregation. Subsequently, they release their intracellular α- and dense granules [22]. Tissues treated with activated PRP will be invaded by PGFs, cytokines, and other platelet lysosomes. More specifically, when the dense granules release their contents, an abundance of pain-modulating 5-HT will be discharged [101]. In PRP, the platelet concentration can be five- to sevenfold higher than in peripheral blood. Therefore, the release of 5-HT from the platelet is astronomical. Interestingly, Sprott et al. [102] reported observing substantial pain reduction following acupuncture and a significant decrease in platelet-derived 5-HT concentrations, with a subsequent increase in 5-HT plasma levels.

In the periphery, endogenous 5-HT is released from platelets, mast cells, and endothelial cells in response to tissue injury or surgical trauma [103]. Interestingly, multiple neuronal 5-HT receptors have been detected in the periphery, confirming that 5-HT can interfere with nociceptive transmission at peripheral sites. The 5-HT system represents a powerful system that can decrease and increase the magnitude of pain following noxious stimulation. Studies have indicated that 5-HT can affect nociceptive transmission at peripheral tissue sites through a variety of 5-HT receptors [104, 105].

In analgesic animal model trials, the potential of PRP analgesic effect was demonstrated by Yoshida et al. [106]. In several clinical studies, the nociceptive and analgesic effects of PRP were discussed. Several studies have indicated little to no pain relief in patients treated for tendinosis pathologies or rotator cuff tears [107, 108]. In contrast, several other studies indicated that PRP reduced or even eliminated pain in patients suffering from tendinosis, OA, plantar fasciitis, and other foot and ankle disorders [109, 110].

The final platelet concentration and the biocellular composition have been identified as key PRP characteristics that contributed to the consistent analgesic effects observed after PRP applications. Other variables included PRP delivery methods, platelet activation protocols, the bioactivity levels of the released PGFs and cytokines, the types of tissues to which PRP was applied, and the type of injury. Notably, Kuffler addressed the potential of PRP in pain relief in patients suffering from mild to severe chronic neuropathic pain, secondary to a damaged non-regenerated nerve. The objective of this study was to investigate whether neuropathic pain would decrease or resolve as a result of PRP's promotion of axonal regeneration and target reinnervation [111]. Strikingly, in treated patients, the neuropathic pain remained eliminated, or reduced, for a minimum of 6 years after the procedure and pain started to decrease within 3 weeks after the surgical PRP application, in all patients.

Recently, similar analgesic PRP effects were observed in the field of postsurgical wound and skincare [112]. Interestingly, the authors reported the physiological aspects of wound pain related to vascular injury and tissue hypoxia, addressing the importance of neoangiogenesis in optimizing oxygenation and nutrient delivery. More pain reduction was noted in PRP-treated patients compared to controls, with significantly higher angiogenetic development in post-PRP-treated patients.

Finally, Johal and co-workers performed a systematic review and meta-analysis and concluded that PRP leads to a reduction in pain following PRP administration in orthopedic indications, particularly in patients treated for lateral epicondylitis and knee OA [10]. Unfortunately, this study did not specify the effects of leukocytes, platelet concentration, or the use of exogenous platelet-activating agents, as these variables affect the overall PRP effectiveness. The optimal PRP platelet concentration that provokes maximal pain relief is yet unknown. In a rat tendinopathy model, complete pain relief was accomplished with a platelet concentration of 1.0 $x10^6/\mu L$, whereas PRP with half this platelet concentration induced significantly less pain relief [106]. Thus, more clinical studies to investigate the analgesic effects of different PRP formulations are needed.

62.3.7 PRP: Angiogenetic Effects

Ideally, the PRP preparations employed in precision regenerative orthobiological therapies allow for the delivery of biomolecules released by a high concentration of platelets, which are ultimately (passively) activated at the target tissue site. As a result, countless physiological cascades are initiated, resulting in on-site immunomodulatory and inflammatory processes, and angiogenesis stimulating healing and tissue repair activities [113].

Angiogenesis is a vibrant, multistep process involving the sprouting and organization of micro vessels from preexisting blood vessels. Angiogenesis progresses due to multiple biological mechanisms, including endothelial cell migration, proliferation, differentiation, and division. These cellular processes are prerequisites to the formation of new blood vessels. They are essential for the outgrowth of preexisting blood vessels to restore blood flow and support the high metabolic activity of tissue repair and tissue regeneration. These new vessels allow the delivery of oxygen and nutrients and the removal of by-products from the treated tissues [114].

Within a diseased and degenerative microenvironment (including a low oxygen tension, low pH, and high lactate levels), local angiogenic factors try to restore angiogenic activities. Following PRP treatment in these MSK pathologies, angiogenic activities are modulated by a balance between pro- and anti-angiogenetic factors. The most important factors are shown in Table 62.5.

Table 62.5 PRP – platelet pro- and anti-angiogenetic factors [15]

Platelet pro-angiogenetic factors	Platelet anti-angiogenetic factors
VEGF	PAI
PDGF-BB	TSP
TGF- β1	TGF- β1
Serotonin	PF-4
MMP-1,-2	Angiostatin
IL-8	TIMPS

Abbreviations: *VEGF* vascular endothelial growth factor, *PDGF-BB* platelet-derived growth factor BB, *TGF-β1* transforming growth factor, *MMP* matrix metalloprotenases, *IL* interleukin, *PAI* plasminogen activator inhibitor, *PF* platelet factor, *TIMPS* tissue inhibitors of metalloprotenases

It has been demonstrated that the overall PRP platelets effects on (neo)angiogenesis is pro-angiogenic and stimulatory [115]. Notably, Landsdown and Fortier [116] reported on the various outcome effects related to the PRP constituents, including intra-platelet sources of numerous angiogenic modulators. Furthermore, they concluded that an increase in angiogenesis contributes to the healing of MSK disorders in areas of poor vascularization, such as meniscal tears, tendon injuries, and other areas with poor vascularity. The administration of PRP, more specifically the delivery of high concentrations of PGFs and other platelet cytokines, can induce angiogenesis, vasculogenesis, and arteriogenesis because stromal cell-derived factor-1a binds to the specific cytokine receptors on endothelial progenitor cells. Another important and essential factor in restoring angiogenic pathways is synergy between multiple PGFs. Richardson et al. [117] demonstrated that the synergistic activities of the angiogenic factors platelet-derived growth factor BB (PDGF-BB) and VEGF result in the rapid formation of a mature vascular network compared to the individual growth factor activities. Most importantly, Giusti and co-workers concluded in a dose defining study that the optimal platelet dose to promote angiogenesis was 1.5×10^6 platelets/μL [64]. Therefore, it is fair to assume that PRP preparations with high concentrations of platelets contain high concentrations of the stimulatory pro-angiogenic PGF VEGF, contributing to significant angiogenetic effects, when compared to PRP preparations with less than 1.5×10^6 platelets/μL.

62.4 Bone Marrow Concentrate (BMC)

The human body has an endogenous system of tissue repair and tissue regeneration through stem cells, as they are found almost in every type of tissue. Clinicians utilizing regenerative medicine applications have a growing interest in using the concentrated bone marrow products, since it is well

acknowledged that BM is a plentiful source of MSCs, progenitors, and other cells residing in the trabecular part of flat and long bones, acquired via appropriately performed BMA procedures [118, 119]. Orthobiological and regenerative medicine treatment options using autologous stem cells can be safely executed by well-trained physicians at point of care.

62.4.1 Bone Marrow Anatomy

The bone is an organ composed of cortical and trabecular bone, cartilage, and hematopoietic and connective tissues. The BM tissue is soft, similar to the peripheral blood, flexible connective tissue comprising the center and the epiphysis of bones, referred to as the BM cavity. In this place, a variety of new blood cells are produced and ultimately released to the peripheral circulation. The bone tissue has an essential role in the structure and protection of the human body. Spongy, or trabecular bone, is composed of a lattice of fine bone plates filled with hematopoietic marrow, fat-containing marrow, and arterial-venous sinusoidal blood vessels. Furthermore, it consists of bone cells at different developmental stages (including pre-osteoblasts, osteoblasts, and osteocytes), collagen fibrils, and calcium and phosphate deposits.

We recognize two categories of bone marrow tissue: the red and yellow marrow. Depending on age, the red marrow is replaced by the yellow marrow. In adults, the red bone marrow is a rich source of bone marrow-derived cells and present in most skeletal system bones of the iliac crest, tibia, spine vertebrae, humerus, calcaneus, ribs, and near point of attachment of long bones of legs and arms. In this well-shielded environment, an estimate of 500 billion cells per day can be produced, in particular erythrocytes, granulocytes, and platelets [120]. For orthobiological applications, the red bone marrow is the preferred type as it contains myeloid and lymphoid stem cells and MSCs.

62.4.2 Bone Marrow Regions

The trabecular bone cavity is subdivided into four region, Table 62.6 [121], according to the model of Lambertsen and Weis. In Fig. 62.5, we illustrate this model which we have adopted and modified for clarification purposes. In general, the bone marrow consists of a hematopoietic component (parenchyma) and a vascular component (stroma). The parenchyma includes hematopoietic progenitor and hematopoietic stem cells (HSCs), which are localized close to the endosteum and around the blood vessels. BM stroma cells, including endothelial cells, are recognized as multipotential non-hematopoietic progenitor cells capable of differentiating into various tissues of mesenchymal origin, including osteoblasts, chondrocytes, tenocytes, endothelial cells, myocytes, fibroblasts, nerves, and adipocytes, as verified in in vitro and partially in in vivo research [122, 123]. The bone marrow's microvasculature includes single layers of endothelium arising in sinusoids, where they also contribute to rolling extravasations of leukocytic cells into and out of the BM tissue structures. The function of the vasculature and BM-derived endothelial cells is that they provide a barrier between the BM compartment as a functional and spatial entity from the extra-lymphoid BM section and the peripheral circulation [9]. The endothelial cells likewise contribute to tissue regeneration, as endothelial precursor cells are essential in improving vascularization of damaged and degenerative tissue cells by the secretion of pro-angiopoietic factors of invading cells [124].

Table 62.6 Bone marrow regions

Bone marrow regions
Subendosteal
Peri-sinusoidal region
Central region
Endosteal region

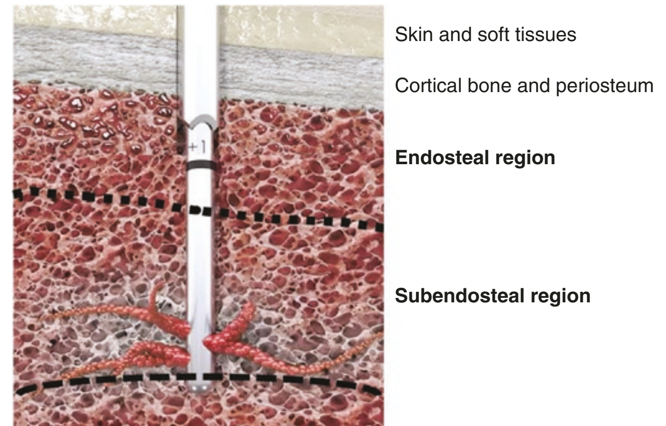

Fig. 62.5 Endosteal and subendosteal regions in a trabecular bone cavity

62.4.3 Bone Marrow Niche

A niche is defined by anatomy and function. Stem cell niches are defined as specific cellular and molecular microenvironments regulating stem cell and progenitor functions. A niche consists of signaling molecules, intercellular contact, and the interaction between stem cells and their neighboring extracellular matrix (ECM). This three-dimensional microenvironment is thought to control genes and properties that define "stemness," including the control and balance between quiescence, self-renewal, proliferation, and differentiation of diverse cell types. Additionally, the microenvi-

Table 62.7 Bone marrow classical niches [26–28, 34]

Bone marrow classical niches
Mesenchymal stem cell niche
Hematopoietic stem cell niche
Perivascular niche
Megakaryocyte niche

ronment provides stem cell autonomous signaling mechanisms [125, 126], and it engages in specific cascades to a stress response [127]. Acquired and prepared BM stem cells from one of the niches and subsequently injected into a totally different microenvironment can potentially differentiate into cell types of this new environment [128]. In Table 62.7, some of the bone marrow niches are given. Since autologously prepared MSCs originate from their specific and original BM niche but are used in other cellular tissue types to treat various pathologies, they can be successfully engaged in tissue repair and regeneration through regenerative medicine application techniques. This is a distinctly different approach in the physiological release of newly formed BM cells because they are retained in the BM cavity until they mature and thereafter released in the vascular peripheral circulation [123].

The role and function of the extracellular matrix (ECM) can be defined as key structural-functional components of cell niches, including soluble factors, cell-cell contacts, and cell-matrix adhesions present in these microenvironments. ECM components include fibrillar proteins, with, among others, collagen fibers, fibronectin, and other filamentous network components. The ECM's mechanical stability is provided by collagen [129]. Other significant ECM components supporting the BM niches are glycosaminoglycans and mainly hyaluronic acid via its receptor CD44. In general, no specific ECM components are identified that maintain MSCs in their immature state, as a niche matrix would do. However, it has become clear that the ECM can regulate MSC differentiation on a solitary basis, indicating potential applications for regenerative medicine applications and tissue engineering.

62.4.4 Stem Cells

Becker, McCulloch, and Till first conducted experiments that led to the discovery of stem cells in 1963 and they produced evidence that these cells were capable of endless self-renewal, which is a fundamental feature of stem cells [130]. A stem cell is a type of cell that is non-specific/specialized in its function. Generally, we recognize two types of stem cells, embryonic and non-embryonic, with two defining properties. Firstly, they have the capacity of self-renewal, therefore giving rise to more stem cells. Secondly, they can differentiate into different lineages under appropriate conditions.

Embryonic stem cells (ESCs) are obtained from 5- to 12-day-old embryos, and they are pluripotent and have a high plasticity as they can differentiate into ectoderm, mesoderm, and endoderm layers, whereas non-embryonic stem cells (non-ESCs) are multipotent, and it appears that they are able to form multiple cell lineages which form an entire tissue, usually specific to one germ layer, e.g., adult stem cells [131].

The capability of stem cell potency, in combination with the relative ease to prepare bone marrow stem cell injectates, is an invaluable property for regenerative medicine cell-based therapies in general and more specifically to treat, e.g., musculoskeletal disorders (MSK-D), chronic wounds, and critical limb ischemia. Friedenstein and colleagues reported first on the isolation of bone marrow–derived stem cells from BM stroma and their incubation in plastic culture dishes, and identified mesenchymal stem cells as colony-forming unit fibroblasts (CFU-Fs) [132]. The BM stroma is made up of a network of fibroblast-like cells and includes a subpopulation of multipotent cells which can generate the mesenchyme, known as the mass of tissue, that develops mainly from the mesoderm of the embryo subpopulation. The cells are referred to as mesenchymal stem cells (MSCs) [133]. The Friedenstein culture method revealed that MSCs can differentiate into several connective tissue cell types [134], described first by Pittenger and associates [135].

62.4.4.1 BMC: Bone Marrow–Specific Stem Cells

The literature articulates BMAs as a heterogeneous mix of cells, referring in most instances to HSCs, MSCs, and mononuclear cells. The roles of platelets, megakaryocytes, and RBCs are seldomly mentioned [136].

1. Hematopoietic Stem Cells

The major function of the bone marrow is to generate blood cells. In adults, marrow-derived HSCs are the principal cells of origin of all mature hematopoietic cell phenotypes, and the process is called hematopoiesis. HSCs are adult stem cells with extensive self-renewal capabilities and can differentiate into specialized blood cells with key roles in some biological activities: control homeostasis balance, immune functions, and response to microorganisms and inflammation. Most HSCs are in a quiescent state within the BM niches. They respond to the signals after the balance of blood cells, or HSC pool, is disturbed from either intrinsic or extrinsic stimuli and signaling processes [137]. Evolving evidence suggests that BM-derived endothelial cells and HSCs, including their progenitor cells, contribute to tissue vascularization. HSCs deliver specific angiogenetic factors, facilitating the incorporation of endothelial progenitor cells into newly sprouting vessels. Several clinical studies have

shown that BM-derived cells contribute to neo-angiogenesis [138, 139]. This should contribute to the clinical discussion of the value of BM-derived HSC and vascular progenitor as they are able to contribute to tissue restoration by accelerating tissue vascularization and regeneration [140].

2. Mesenchymal Stem Cells

In recent decades, physicians performing orthobiological/regenerative medicine procedures have been more interested in the potential of BM-MSCs than of HSCs. Imaginable reasons for this particular interest in MSCs might be due to recently published expert opinions: the in vivo ability of MSCs to migrate into tissues, their sturdy regenerative and reparative properties, and MSC-mediated immunomodulatory actions. These typical characteristics and their particular mode of actions enable conceivable BM cell-based treatment options [141, 142]. MSCs do not express significant histocompatibility complexes and immune-stimulating molecules, leading to graft rejection. Likewise, a rapid development in clinical outcome reporting, with a better understanding of BM tissue molecular biology, improved bone marrow aspiration techniques and, and preparation methods, has increased the interest and indication for autologous BM stem and progenitor cell therapies.

MSC function: MSCs are multipotent stem cells which can be obtained from various adult tissues, like the BM stroma, adipose tissue, synovium, periosteum, and trabecular bone. Typical features are their ability for self-renewal, defined as sustaining biological pathways and mechanisms to preserve the undifferentiated stem state, and the regulation of lineage-specific differentiation [143]. Although the number of MSCs represents only a small fraction of non-hematopoietic, multipotent cells of the bone marrow (0.001–0.01%), understanding these unique cells has taken great strides forward. Under appropriate conditions and an optimal microenvironment, MSCs can differentiate into various mesodermal lineages like osteoblasts, chondrocytes, endothelial cells, adipose tissue, and smooth muscle cells [144]. These MSC proficiencies have led to the use of MSC as a potential strategy for treating various diseases, since they encourage biological processes, for example angiogenesis, cell proliferation, and cell differentiation [145]. Furthermore, they synthesize cytokines and trophic mediators which participate in tissue repair processes, immune modulation, and the regulation of inflammatory processes [146]. Based on the above characteristics, it can be assumed that MSCs are capable of instituting a regenerative microenvironment at the site of release and improving various cell recruitment, cell signaling, and differentiation of endogenous stem cells, with the potential to instigate tissue repair in a variety of disease states.

62.4.4.2 BMC: Harvesting and Preparation

Several groups have mentioned some considerations when performing BM harvesting procedures, addressing a variety of factors that have an impact on patient comfort and the quality of the harvested BM. Emphasis was given to procedural safety issues when using harvesting needle systems, level of experience of the operator, the choice for concentration technology and centrifugation devices, and pain management [147]. Autologous regenerative medicine BM-MSC applications may range from harvesting a low volume of BM and direct, unprocessed, tissue injection to the use of centrifugation protocols to concentrate and filter the BMA prior to injecting it in patients [148]. Various bone marrow harvesting systems are available on the market, each with their own proprietary design characteristics, and thus marrow cellular dynamics when extracting marrow. Potentially, different BM needle design features might affect the quality and cell viability of the harvested marrow tissue, as well as the cellular yields, before and after processing [149, 150]. Bone marrow aspiration anatomical sites: As MSCs represent a small population of BM cells [135], it is of critical importance to choose a BMA site that will yield the most MSCs. BM is relatively easy to harvest, largely available, and dispensable. Obviously, it is important that the BMA procedure is performed impeccably to obtain an optimal quality of viable BM tissue [133, 151]. In humans, the most common anatomical location to obtain BM is the iliac crest, but other BMA sites have been utilized [8]. Recently, McDaniel and co-workers, reported that all studied anatomical bone marrow harvesting locations contained MSCs, but the iliac crest was the most abundant source of MSCs [120], in particular posterior superior iliac spine (PSIS) [152].

62.4.4.3 Image Guidance for Bone Marrow Extraction

To perform BMC procedures, a certain volume and quality of bone marrow tissue are required. The aspiration volume is contingent on the processing volume of the BMC concentration system that is being used. It is imperative to precisely locate the BM donor site, as MSCs are mostly located in the endosteal and subendosteal marrow region and some are present around the blood vessels [127, 153, 154]. The precise delivery of local anesthetics and safe trocar placement before marrow extraction are accomplished by using image guidance [155]. In the following section, we focus on the posterior super iliac spine (PSIS) sites, as it is the most frequently reported anatomical site for BMA.

62.4.5 BMAC Harvesting: Ultrasound Guided

When the PSIS is targeted, patients are positioned in the prone position, while avoiding lumbar lordosis. The sono-

graphic assessment uses a portable ultrasound system with a 5-2 low-frequency curvilinear transducer positioned in a transverse plane over the hyperechoic bilateral sacral cornual, with the patient lying prone and the monitor screen in the line of sight of the operator. The probe is then translated contralaterally from the physician, keeping the hyperechoic sacrum visualized. Next, the probe is translated proximally, with the hyperechoic ilium coming into view, while maintaining the hyperechoic sacrum, until the most superficial depth of the ilium is reached, known as the PSIS, contralateral to the examiner [156]. After identification of the PSIS, the most superficial depth is confirmed in both transverse and longitudinal orientation (Fig. 62.6). With the probe in the transverse plane at the PSIS, the slope of the iliac wing is noted for correct angulation of the BM trocar, and the most superficial depth of the PSIS is brought under the most medial aspect of the ultrasound probe. Using a sterile marker, a mark and directional line are made in both parallel and perpendicular orientations to form an intersection at the most superficial depth of the PSIS. This mark is maintained on the patient during skin preparation prior to the introduction of the BM trocar, and a superficial wheal of local anesthetic is placed at the point of planned trocar skin entry. Following the local antiseptic measures, sterile ultrasound gel is applied at the marked area, and a sterile probe cover is applied to the 5–2 MHz curvilinear array transducer. Typically, a mixture of local anesthetics is injected around the PSIS cortex and periosteal sleeve, under continued sonographic guidance, making sure to "walk off" the PSIS in four directions (superiorly, medially, laterally, and inferiorly), confirmed by sonographic guidance. The trocar is then introduced, using either a manual force that is perpendicular or slightly lateral to the patient, at 9–12 counterclockwise-clockwise rotations, or a mallet. The next step of the procedure is subject to the implementation of the instructions for use provided by the manufacturer of the aspiration harvesting system.

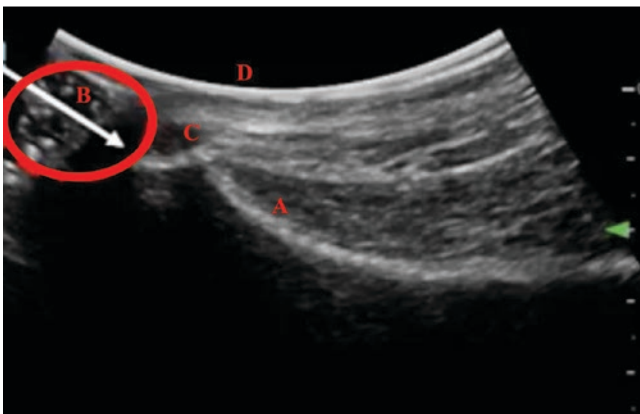

Fig. 62.6 Ultrasound image of the right PSIS, with the probe in transverse plane. A indicates the wing of the PSISI; B is the angulation of the trocar; C marks the superficial depth of the PSISI below the skin (D)

62.4.6 BMAC Harvesting: Fluoroscopic Guided

After proper patient positioning, the fluoroscopic equipment is installed to optimize the positioning for fluoroscopic imaging, using ipsilateral or contralateral oblique beam angulations for viewing the targeted PSIS site. The perpendicular fluoroscopic approach requires a beam angle around 15° ipsilateral to the PSIS entering laterally with angulation toward the sacroiliac joint. This angle will view the lateral ilium outer wall, and a needle is directed anteromedially. Fluoroscopic images support in positioning the tip of the trocar above the target area for entering the PSIS. The parallel fluoroscopic approach results in viewing down the PSIS table, at a 25° contralateral oblique beam position. This results in a classic view of the "teardrop" (Fig. 62.7).

Imaging can confirm the entry point into the PSIS table and visualize the angle through the cortex, allowing for safe trocar advancement in the BM cavity, at the tick part of the ilium bone [157]. Using proper fluoroscopic techniques, the parallel approach technique allows for a safe deeper marrow penetration. However, always, regardless of the approach,

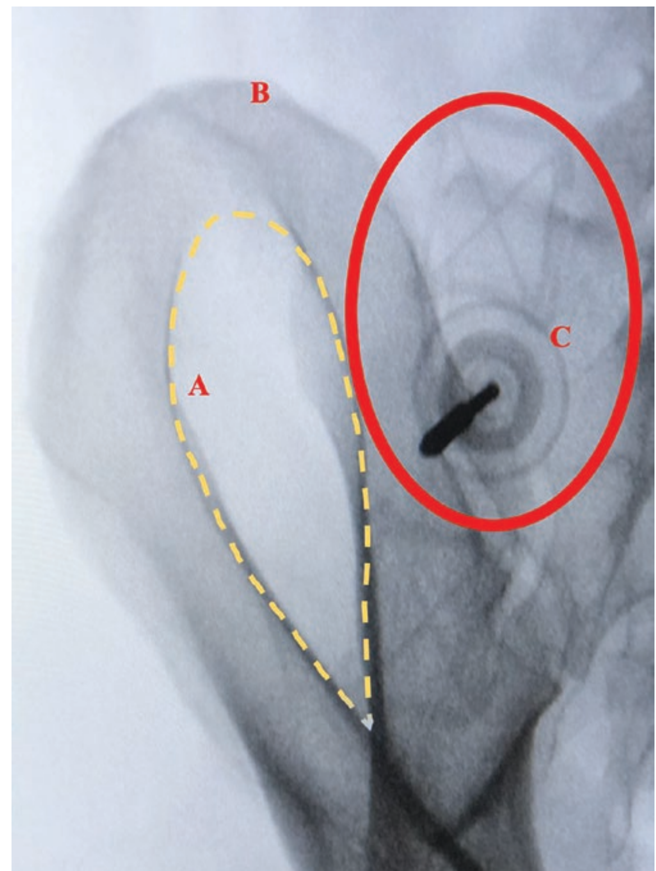

Fig. 62.7 The parallel fluoroscopic approach, viewing down the PSIS table (B), at a 25° contralateral oblique beam position. This results in a classic view of the "teardrop" (A). (Outline of the medial and lateral borders, in yellow). The tip of the BMA trocar and handle (C) marks the entry site in the marrow cavity

avoid increased manipulation and tissue trauma using the sharp trocar, as this will increase the risk for neurovascular injury, bleeding, tearing of lateral gluteal muscle origins, and post-procedural pain.

62.4.6.1 BMC: Immunomodulatory Effects

For MSCs to become "immunosuppressants," they need to be triggered by inflammatory cytokines, and the inflammatory environment is then a crucial factor for MSCs to exert their immunomodulatory effects. These are wielded by blocking apoptosis of native and activated neutrophils, aside from decreasing neutrophils from binding to vascular endothelial cells and mobilizing neutrophils to the area of damage [158]. However, the mechanisms by which MSCs are mobilized and recruited to damaged sites are not known. In addition, how they survive and differentiate into distinct cell types is still not clear. Once MSCs have been applied to the microenvironment of injured or degenerated tissues, many factors stimulate the release of many growth factors by MSCs. These growth factors stimulate the development of fibroblasts, endothelial cells, and tissue progenitor cells [159]. It is credible to state that the use of MSCs and their potential in immunomodulation in regenerative medicine applications hold great promise [160, 161].

62.4.6.2 BMC: Angiogenetic Effect

MSC paracrine trophic factors are potentially important in maintaining endothelial integrity and promoting angiogenesis through their ability to regulate endothelial cell proliferation and ECM production [162]. Furthermore, endothelial cell permeability is reduced, and MSCs inhibit interactions between leukocytes and endothelial cells [163]. Apart from MSC trophic factors, fibroblasts have fundamental functions in maintaining tissue integrity and promote tissue healing through their secretion of cytokines that support ECM building. These endothelial and angiogenetic capabilities have been demonstrated in clinical studies addressing chronic wound healing [164, 165] and recovery from post-myocardial infarction [166].

62.4.6.3 BMC: Tissue Repair Processes

BMACs are heterogeneous cell compositions that include BM-MSCs, making them endogenous cell sources for regenerative medicine repair treatments. They act by reducing cell apoptosis, fibrosis, and inflammation and activating cascades that lead to cell proliferation [167, 168]. In addition, BM-MSCs have the potential to differentiate into multiple cell lineages, including osteoblasts, adipocytes, myoblasts, epithelial, and neuronal cells [144]. They also contribute to angiogenesis via paracrine and autocrine pathways. Equally important, BM-MSCs are contributors to immunomodulatory actions independent of immune-specific cells, which participate in the inflammatory phase of wound repair [161, 169]. Moreover, BM-MSCs support the recruitment of cells to neoangiogenic treatment sites to accelerate local revascularization [170]. Kim et al. demonstrated that in the absence of an adequate scaffold, the survival rate of BM-MSCs and their reparative and differentiation capacity to enhance healing are jeopardized [171]. Although tissue harvesting, specimen preparation, and mechanism of action are different for PRP and BMCs, studies have shown that they can complement each other [172, 173].

62.4.7 BMC Trophic Effects Mediated by PGF

PRP platelet growth factors are crucial proteins that are involved in the BMC reparative processes and their role is to stimulate the various MSC capabilities, activities, and reparative functions, a phenomenon termed PRP trophic effects. The diversity of PDGFs and other cytokines involved in BMC trophic processes can initiate tissue repair by decreasing cell apoptosis and anabolic and anti-inflammatory effects, and by activating cell proliferation, differentiation, and angiogenesis via paracrine and autocrine pathways [174, 175]. Explicitly in OA treatments, PDGF plays a specific role in regenerating cartilage and maintaining homeostasis via MSC proliferation and inhibition of IL-1-induced chondrocyte apoptosis and inflammation [176]. Also, three TGF-β isoforms are active in stimulating chondrogenesis and inhibiting inflammation, and they express their ability to promote MSC-associated tissue healing via inter-molecular actions [174]. MSC trophic effects are associated with PGF activity and the secretion of reparative cytokines. Ideally, all of these cellular factors should be present in the BMA treatment vials and delivered to tissue injury sites to promote optimal MSC-associated therapeutic tissue healing [175].

Combining BMC and PRP: There is minimal information available on the presence or concentrations of PGFs in BMCs, or the ideal ratio needed to support BM-MSC trophic actions. Some clinicians combine high PRP concentrations with BMACs to have potentially more biologically active graft, projected to optimize regenerative medicine treatment outcomes [177]. However, there are minimal safety and efficacy data available that indicates that combining high PRP concentrations with BMAC is a more effective treatment option. Therefore, we believe that manipulating BMMSCs by priming them with high platelet concentrations may not be indicated at this stage.

62.5 Adipose Tissue Concentrate (ATC)

Aside from PRP and BMC preparations, adipose tissue (AT) has been used as a cell-based therapy in orthobiological and regenerative medicine procedures to create an adipose tissue

concentrate (ATC), harvested and prepared at point-of-care in an office setting. Autologous AT is a heterogeneous biological source of various cellular tissue components. Furthermore, concentrated adipose tissue provides clinicians with a physiological 3D multicellular scaffold, including adipose stem cells (ASCs) and stromal cells. Both autologous and allogeneic ATCs have been employed in clinical trials to treat conditions such as lipoatrophy, muscular dystrophy, myocardial infarction, stroke, and spinal cord injury [178, 179]. ATCs have demonstrated to be effective in the treatment of MSK disorders and other regenerative applications, comparable to MSCs originating from BMCs. Like other MSCs, ASCs can differentiate into cells of mesodermal (osteoblasts, adipocytes, and chondrocytes), endodermal (hepatocytes, pancreatic cells), and ectodermal (neurons) primary layers [180]. These characteristics are of high interest when treating MSK disorders like osteoarthritis and chronic tendinopathies.

62.5.1 Adipose Tissue Structure

Adipose tissue is a highly vascularized connective tissue, abundantly present throughout the human body. White AT (WAT) is responsible for energy storage and plays a pivotal physiological role in maintaining metabolic homeostasis in the body by releasing several adipocytokines, growth factors, and cytokines that may act in an endocrine or paracrine fashion [181]. Brown AT (BAT) plays a significant role in thermogenesis via the actions of uncoupling protein 1. BAT cells present the ability to disperse energy by producing heat to ensure body temperature regulation, rather than storing it as triglycerides [182].

62.5.2 Adipose Tissue as a Source of Stem Cells

In 2002, Zuk et al. performed the first characterization of adult stem cells, isolated from lipoaspirates, demonstrating that ASC derived from WAT lipoaspirate exhibits MSC properties, like plastic-adherent, multipotency, and differential capacity [183–185]. AT MSCs have a high proliferation capacity and multilineage cell differentiation potential capable of differentiating into adipogenic, chondrogenic, myogenic, osteogenic, and neurogenic cells [186]. These AT-specific characteristics, combined with an abundance of MSCs when compared to MSCs derived from bone marrow [187, 188]. Therefore, ATs prepared form WAT have great potential in clinical orthobiological tissue repair applications [189, 190]. Furthermore, Yun et al. described the AT MSC-mediated effects on the reduction of proinflammatory cytokines, chemokines, cellular apoptosis, and collagenases [191]. Moreover, AD-MSCs have been shown to be immune-privileged [192]. AT has been one of the most studied tissues in the last decade [193–195] and they are increasingly popular. Practitioners value the high MSC content in ATC and they consider harvesting of subcutaneous WAT beneath the skin as a relatively easy procedure. Consequently, physicians might consider that MSCs from ATC have distinct advantages over "conventional" BMC preparations for orthobiological applications.

62.5.2.1 Stromal Vascular Fraction (SVF)

The use of AT in regenerative medicine is based on the separation of the vascular stroma contained in ATC, allowing for access to AD-MSCs [196, 197]. The isolation of AD-MSCs from WAT involves the separation of adipocytes from the remaining adipose cells of the SVF. SVF is a heterogeneous collection of cells contained within adipose tissue and can be isolated from fat using different disruption techniques, enzymatic digestion, or mechanical emulsification (ME).

Various techniques, including centrifugation (density gradient layer separation), are available for preparing a viable biological specimen to initiate SVF production. Centrifugation techniques have proven to be an effective means to safely wash, rinse, eliminate the infranatant-extracellular fluid, separate free lipids and residual oil, and prepare concentrated adipose tissue. In Fig. 62.8, the final processed adipose tissue is shown.

AD-MSCs and SVF cells, both contained in the SVF, meet the four criteria for MSCs as defined by the International Society for Cellular Therapy (ISCT) [198]. The presence of these cells can be measured by laboratory techniques, including flow cytometry techniques, as each cell has their own unique cell surface marker [199]. In Table 62.8, the heterogeneous SVF cellular distribution is shown.

62.5.3 Enzymatic Digestion vs. Mechanical Emulsification

Enzymatic digestion techniques use enzymes (collagenase) to isolate stromal and MSCs from adipose tissue by digesting the peptide bonds in the collagen of WAT with the destruction of extracellular structures. Centrifugation techniques are employed to separate the floating adipocytes from the pelleted SVF, following good manufacturing practices regarding closed, sterile, and safe isolation processes [200]. In these preparation protocols, a combination of enzymatic digestion and incubation/agitation has been identified, producing an adipose-derived cellular SVF (AD-cSVF). Freshly isolated SVF can directly be applied, without the need for further cell separation or in vitro expansion. AT-MSCs constitute as much as 1% of SVF cells compared with the 0.001–0.002% of BM-MSCs in bone marrow [201]. Furthermore,

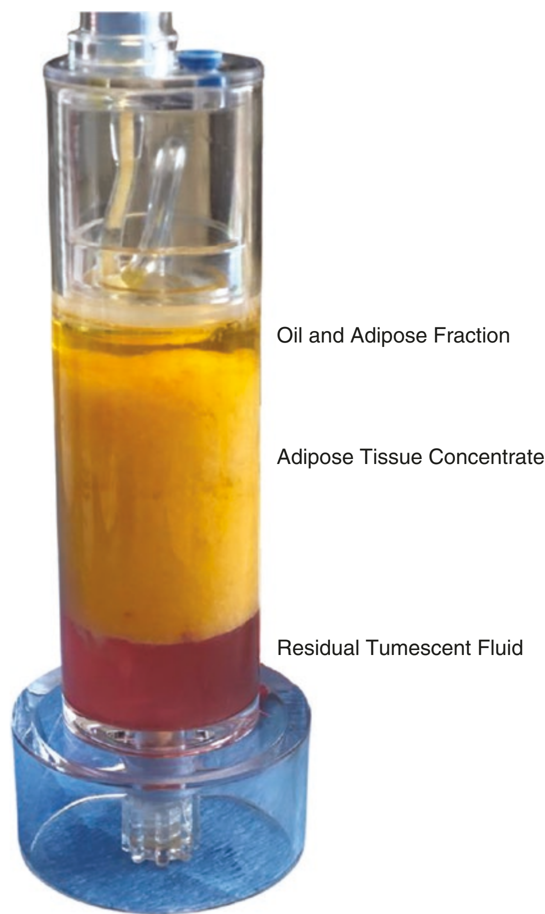

Fig. 62.8 Centrifugated density separation of adipose tissue, showing on top the oil and adipose fraction. The middle layer is the ATC, and at the bottom of the concentration device (Progenikine® Adipose Concentration System, EmCyte Corporation, Fort Myers, FL, USA, with permission) is the residual tumescent fluid

Table 62.8 SVF Cellular distribution

SVF Cellular distribution
15–30% Stromal cells: AD-MSCs Pre-adipocytes Fibroblasts
35–45% Hematopoietic-lineage cells: Erythrocytes Platelets Neutrophils Lymphocytes Monocytes/macrophages
1–15% Hematopoietic stem and endothelial progenitor cells
3–5% Pericytes
10–20% Endothelial cells
5–15% Smooth muscle cells

Abbreviations: *AD-MSCs* adipose mesenchymal stem cells

detrimental erythrocytes are usually removed using a lysis buffer with a standard enzymatic processing protocol compared to a BMC product, which contains a significant number of erythrocytes [200]. In current FDA guidelines, enzymatic cellular prepared SVF products fall into the "more than manipulated" category and require specific clinical trials to examine and report the long-term safety and efficacy of such products in human clinical uses.

Another method to produce SVF refers to the definition of adipose-derived tissue SVF (AD-tSVF). This method addresses the adipose stromal population within a bioactive scaffold, or extra cellular matrix tissue, as described by Alexander. AT, acquired via lipoaspiration techniques, can be concentrated to an ATC by centrifugation and is thereafter subject to mechanical disruption by a process termed mechanical emulsification (ME) (Fig. 62.9). Hereafter, the final product is often termed microfat or nanofat, since it is composed of both cellular and native structural fragments [202, 203]. Therefore, emulsified AD-tSVF produces not a 100% concentrated cellular product when compared to AD-cSVF. Ultimately, after ME of ATC, the final AD-tSVF treatment specimen is injected into diseased tissue structures enabling the AD-MSCs to repair damaged and diseased tissues.

An advantage of this ME method is that prepared AD-tSVF offers the ability to provide a bioactive cellular tissue matrix after tissue application. Additionally, anti-inflammatory or immunosuppressive factors are secreted, capable of exerting immunomodulatory effects [204]. Noteworthy, AD-tSVF preparations and their subsequent applications are approved by the FDA.

62.5.3.1 Adipose Immunomodulatory Effects

Several studies have compared the immunomodulatory abilities of AD-MSCs and BM-MSCs and have shown that they exhibit similar effects when used in autoimmune diseases and chronic inflammatory conditions [205–207]. AD-MSCs can regulate the immune system directly via cell-cell communication and indirectly through the secretion of soluble mediators, growth factors, and extravascular vesicles [208, 209]. In adipose tissue, AD-MSC interact with numerous cell types, including immune cells, endothelial cells, pre-adipocytes, hematopoietic cells, nerve cells, endothelial cells, and pericytes surrounding the blood vessels. AD-MSCs and immune cells can interact because they can regulate and influence the activity of T cells, B cells, and macrophages in vitro and in vivo [210]. Indirect cell-cell communication is instigated by AD-MSCs when they secrete soluble mediators and extravascular vesicles (exosomes and micro vesicles) that are known to have therapeutic effects in regenerative medicine [211]. The most cited soluble mediators are pro-inflammatory and anti-inflammatory cytokines, adipokines, antioxidative, pro-angiogenic, anti-apoptotic, growth factors (like, VEGF, FGF, TGF), and specific interleukins (IL-6, IL-7). Currently, the clinical production of SVF to acquire AD-MSCs is subject to investigations addressing their

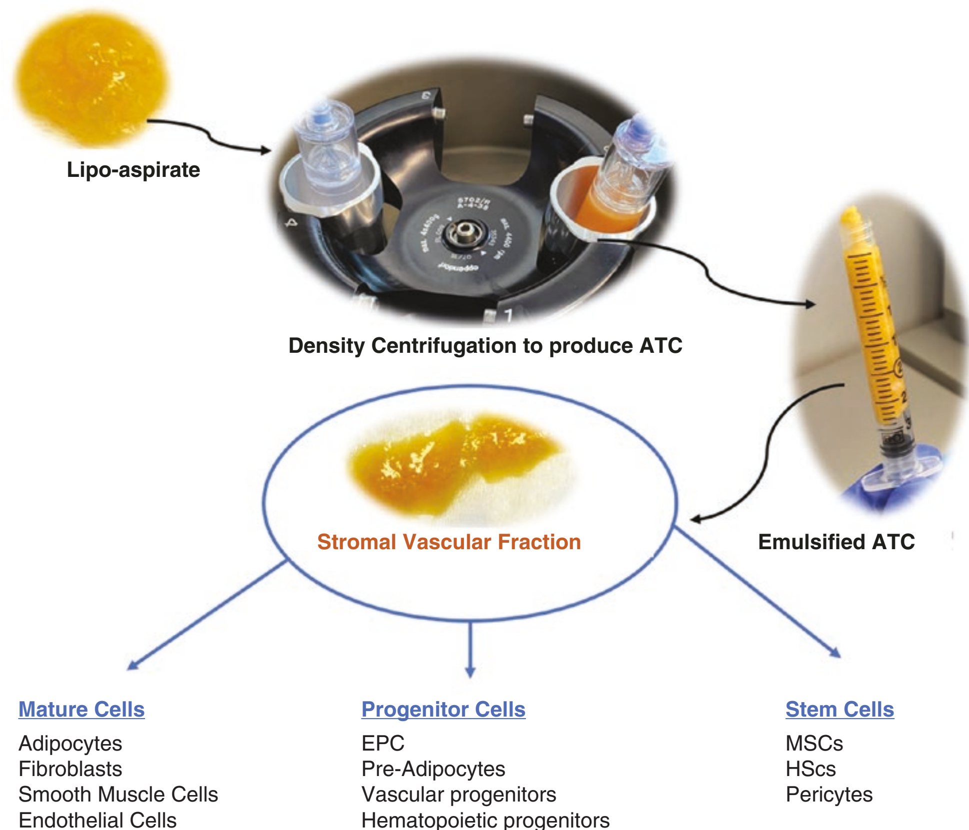

Fig. 62.9 SVF production steps. Adipose tissue is harvested following lipoaspiration and subsequently processed in a centrifuge for density cellular layering. After centrifugation, the ATC can be subject to ME before application as an orthobiological preparation. After EM, SVF cellular components will be liberated from the concentrated AT. This heterogeneous mixture is characterized as mature, progenitor stem cells

immunomodulatory potential in regenerative medicine. Critical aspects in these studies are the ability to develop standardized preparation protocols to ensure effective and safe use in orthobiological procedures.

62.5.3.2 ATC: Angiogenetic Effect

ATs have been intensely studied for the treatment of multiple conditions as they have great potential in orthobiological and regenerative medicine. AD-MSCs show paracrine activity and exhibit differentiation potential toward different cell lineages (adipogenic, osteogenic, chondrogenic, and myogenic lineages), while providing immunosuppressive properties and low immunogenicity [212]. AT produces and secretes various angiogenic factors such as angiopoietin-2 (Angpt2) and VEGF, as well as adipokines such as leptin and adiponectin, which influence and modulate angiogenesis and the vascular structure [213]. This suggests an autoregulatory function for angiogenesis in AT. Interestingly, precursor cells in blood vessel wall have been identified capable of differentiating into endothelial cells and/or adipocytes in WAT [214]. They concluded a high adipogenic potential, linking EC and adipocytes in terms of interchangeability based on cell-cell interactions, enabling them to participate in the formation of angiogenesis and neovascular structures. Therefore, AD-MSCs might affect the growth of capillary networks which are required in adipose tissue enlargement [215]. Furthermore, by enhancing angiogenesis and vasculogenesis, AD-MSCs promote neovascularization, which is fundamental in the treatment of tissue repair and post-(ischemic) injuries. These specialized characteristics of ATC were demonstrated by Miranville et al., revealing the expression of CD34 and CD133 of AD-MSCs which can differentiate into endothelial cells, contributing to revascularization [189]. Not only do AD-MSCs stimulate angiogenesis through differentiation into epithelial cells but also through paracrine activity, releasing angiogenic factors.

More specifically, the cellular components of SVF are rapidly restored to form new vessels in diseased tissue structures following orthobiological injections [216]. Neovascularization is further stimulated by stromal cells through the release of VEGF, TGF-b, and hepatocyte growth factor (HGF) [217]. Macrophages have demonstrated to be important cells in SVF for the proper structural organization of new blood vessels [216].

62.5.3.3 ATC: Tissue Repair Processes

Tissue repair processes following ATC and AD-tSVF preparations for orthobiological indications are based on their stromal, multipotent, and hematopoietic cell populations.

In addition, AD-tSVF can produce an assortment of angiogenic, hematopoietic, and anti-apoptotic factors that

further expedite tissue repair (regeneration) via autocrine and paracrine actions [179].

SVF has the capacity to attain positive treatment outcomes through multiple cell components and tissue scaffold interactions with the extra cellular matrix (ECM).

The ECM is known as a potent scaffold in many tissues and accelerates the capability of regenerative functions by nearby cells [196]. The ECM encompasses structural proteins excreted by fibroblast, such as collagen, fibronectin, and elastin. A typical ECM characteristic is its ability to interact dynamically with integrin proteins on adhesive cells, triggering signaling pathways and changes in cell activity [218]. Furthermore, the ECM contributes to the growth of vascular infrastructure during angiogenesis [219]. Since the SVF comprises matrix-secreting fibroblasts and other stromal cells, the clinical application of ATC and SVF is possibly beneficial to various tissue types that benefit from a three-dimensional (3D) scaffold, like the ECM of tendons, which is composed of collagen and a smaller fraction of elastin embedded in a hydrated proteoglycan matrix [220].

ATC and AD-MSCs have been recognized as effective regenerative treatment modalities over the past decade. Aside from their capacity to differentiate into a variety of mature cell types, the stromal fraction within the SVF, including fibroblasts and stem cells, stimulates angiogenetic processes and the ECM secretes, among others, collagen proteins [221]. It has been suggested that fibroblast-derived ECM components are essential for the development of blood vessels and that angiogenesis necessitates synergy between stromal and endothelial populations [222]. Therefore, regenerative mechanisms demand synergy between angiogenesis and the synthesis of ECM proteins, founding a suitable milieu for tissue healing. Another tissue repair process is mediated by the presence of monocytes and macrophages in SVF. Approximately 5–15% of these cells are anti-inflammatory macrophage phenotype M2, an important component in controlling the environment for regeneration [223]. Additionally, 10–15% of the SVF comprises lymphocytes, including regulatory T cells contributing to tissue immune responses [223].

It appears that the heterogeneous composition of tissue SVF has distinct advantages. The interplay of controlled inflammation with immunomodulatory properties of AD-MSCs, as precursors to ECM formation and angiogenesis, provides the necessary constituents for cellular and musculotendinous repair processes and promotes the restoration of functional tissue.

62.5.3.4 Adipose Tissue Harvesting

Liposuction is the removal of subcutaneous fat by which it is possible to obtain the adipose tissue for autologous fat transplantations and for the preparation of ATC, for ASCs therapeutic treatments. The procedure is executed by means of aspiration cannulas, introduced through small skin incisions, assisted by suction. Its basic principles have been elaborated by Illouz, who was the first to introduce the modern, safe, and widespread method of liposuction with a blunt-tipped cannula as well as subcutaneous infiltration to facilitate adipose breakdown and aspiration [224, 225]. The procedure preserves neurovascular structures while maintaining fluid balance, with minimal patient discomfort [226]. In 1985, the tumescent liposuction technique was introduced by Klein [227]. Later, Coleman introduced a new three-step technique to decrease trauma to adipose tissue following liposuction: manual lipoaspiration under low pressure, centrifugation for 3 min at 3000 rpm, and 3D matrix reinjection [228]. In general, lipoaspiration techniques should not affect cell viability and consequently the yield of ASCs [198, 229]. Harvested WAT is transferred to a concentration device using minimal processing steps for autologous concentrated fat grafting preparations and ultimately ASC functionality (Fig. 62.10). An overview of adipose harvesting steps is given in Table 62.9.

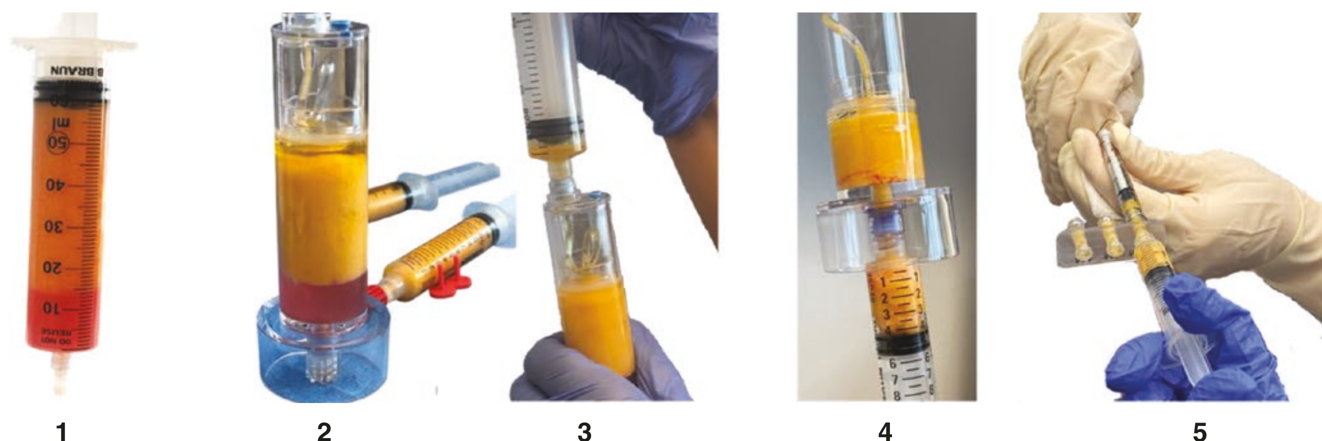

Fig. 62.10 ATC preparation techniques reviewed. After harvesting of AT, the extracted AT is racked and decanted from the syringe (1). After centrifugation (2), the oil (3) and tumescent fluid are removed, and subsequently the ATC is collected (4). Before injecting ATC, mechanical emulsification will size the ATC and break down the adipocytes (5)

Table 62.9 ATC preparation steps

A. Tumescent fluid preparation: a sterile NaCl solution consisting of anesthetics (lidocaine for pain relief and epinephrine for blood vessels to constrict to minimize RBC contamination in fat tissue during harvesting).
B. Tumescent injection: via small skin cuts, a thin blunt injector needle is injected in the target adipose harvesting area.
C. Waiting time: reports indicate to wait at least 20 minutes before starting the fat-harvesting procedure. This time is needed for the fluid to cause the injected area to swell and stiffen, supporting in easy fat removal.
D. Adipose harvesting: with a dedicated harvester cannula, fat tissue is harvested using liposuction; by applying manually negative pressure to a collection syringe, fat is removed from the area that was injected with tumescent fluid.
E. Racking and decanting: syringes filled with harvested fat are placed in a rack, with plunger in upward direction. After the adipose harvesting, leave all syringes in the rack for 10–15 minutes, with the luer connection of the syringe capped. Decant the supernatant (tumescent fluid) by removing the cap, until adipose tissue starts to block the luer.
F. Transfer the decanted fat into a disposable processing device and place it in a dedicated centrifuge to concentrate the AT specimen.
G. Centrifugation protocol: density layer separation by centrifugation, producing ATC. Follow the instruction for use of the preparation device to extract ATC.
H. Mechanical emulsification: a method to emulsify the ATC, by moving the two syringes back and forward through a restraining device to size the ATC, making it suitable for tissue injection.

62.5.3.5 Bone Marrow MSCs Versus Adipose MSCs

Adult MSCs are undifferentiated multipotent cells characterized by the capacity for self-renewal and the ability to differentiate into various cells of mesenchymal origin, including adipocytes, chondrocytes, myocytes, and osteoblasts, when exposed to specific growth signals [13], and the ISCT proposed clear criteria for the definition of both BM and AT MSCs [223].

Comparing AD-MSCs and BM-MSCs, the latter have been the most extensively used and investigated MSCs. However, some practitioners consider limitations with extracting BM-MSCs. Cited limitations are the possible pain and morbidity following harvesting and the limited number of MSCs that can be obtained, as only a relatively small amount (0.001–0.01%) of harvested BM cells are MSCs [135]. These issues have led for the search of alternative and comparable sources for MSCs. Abundant numbers of AD-MSCs have been reported to be easily isolated from adipose tissue by a minimally invasive procedure [190]. In addition, AT can be harvested from multiple sites [230–232]. Various studies indicate that BM-MSCs and AD-MSCs have comparable characteristics when it comes to CD markers and morphology [223], and like BM-MSCs, AD-MSCs can undergo multi-lineage differentiation, including osteogenic, chondrogenic, adipogenic, cardiomyocytic, hepatic, and neurogenic differentiation [233]. It has been postulated that the capability of proliferating and differentiating into different mesenchymal lineages make AD-MSCs a promising less-invasive alternative to BM-MSCs for cell-based therapeutic applications [234]. Klar et al., a cellular comparison was performed between BMC and SVF and a conclusion was made that the mononucleated fraction of the SVF is richer in stromal cells (15–30% of all cells) [235]. However, most of these studies compared AD-MSCs and BM-MSCs obtained from different individuals [236], lacking donor-matching MSCs from BM and AT tissues, and they were studied in MASC expansion studies. Furthermore, there is a lack of standardized methods and quality controls tests to translate scientific findings from basic science into the standard of orthobiological applications.

In an in vitro study, Mohamed-Ahmed and co-workers compared the properties of BM-MSCs and AD-MSCs, acquired from the same individuals [13]. They reported comparable multi-potencies, morphology, and immunophenotyping for both MSC types. Nevertheless, their tissue-specific differentiation capacity varied. BM-MSCs were superior to AD-MSCs in terms of osteogenic and chondrogenic differentiation, while AD-MSCs had higher proliferation and adipogenic potential. Furthermore, other reports have also concluded that MSCs preferentially differentiate into cells of their native microenvironment [237, 238].

62.6 Summary

Autologous PRP can be easily prepared from whole blood and can ultimately secrete multiple growth factors and other cytokines for regulating various physiological activities. These platelet constituents induce tissue repair and are capable of stimulating proliferation and differentiation of different stems cells in MSK injury models and have been used safely and effectively for decades. Current literature supports the use of PRP in early OA and other indications. Unfortunately, we are still in search for consensus of appropriate bioformulations and preparation standards to treat different maladies. PRP treatments require considerably less resources when compared to MSC cellular-based therapies.

Following PRP treatments, autologous progenitor and MSC stem cell-based therapy have emerged as an alternative strategy in MSK disorders to overcome the limitations and consequences of more invasive treatment procedures. These limitations include (post-surgical) treatment morbidity, risk of infection, wound healing disturbances, and hemorrhage [239]. Despite their safety, regulatory complexities have materialized to implement MSC cell-based therapies, with more restrictions to the use of adipose tissue, making these treatments less available for most patients. Although autologous orthobiological interventions demonstrate very promising results, some (competitive commercial) organizations

consider them experimental, with unproven cost/efficacy data and treatment durability.

High expectations on the performance of all three discussed orthobiologics will be met after clarifying scientific barriers, through a clearer understanding of the underlying mechanisms of action. In addition, comprehending the various crucial clinical features of MSK pathologies, that potentially can be treated with orthobiologics, will allow for the improvement of orthobiological product definitions and application procedures. Combined with patient stratifications according to biological and clinical criteria, these aspects together will increase the efficacy of PRP, BMC, and ATC treatments and contribute to move the field of management of musculoskeletal and sports lesions forward, potentially increasing the utilization and acceptance of autologous orthobiological treatment modalities.

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