ABSTRACT

Regenerative medicine is one of the fastest-growing areas of human and veterinary medicine in restorative medicine, orthopedics and neurology. Platelet-rich plasma (PRP) and bone marrow (BM) are one of the fastest growing regenerative methods for the treatment of various degenerative diseases of bones and soft tissues. Platelet-rich plasma (PRP) and bone marrow (BM) are the sources of a number of growth factors and proteins that accelerate the regeneration of damaged tissues and thus make them applicable to acute and chronic traumas.

In modern regenerative medicine, there are numerous protocols for standardization and production of platelet-rich plasma (PRP) and bone marrow (BM).

In this article, we review the different principles and methods for obtaining Platelet-rich plasma (PRP) and Bone marrow (BM) based on available literature.

Key words: Platelet-rich plasma (PRP), bone marrow (BM), principles, methods, preparation.

Introduction

Regenerative medicine is one of the fastest-growing areas of human and veterinary medicine in the area of restorative medicine, orthopedics and neurology. Platelet-rich plasma (PRP) and bone marrow (BM) are one of the fastest growing regenerative methods for the treatment of various degenerative diseases of the bones and soft tissues. PRP and BM are the source of a number of growth factors and proteins that accelerate the repair of damaged tissues and thus, make them applicable to acute and chronic traumas.

In modern regenerative medicine, there are numerous protocols for standardization and production of PRP and BM. This article discusses the different principles and methods of preparing PRP and BM based on a literature review.

The most commonly used regenerative therapies for the treatment of osteoarthritis (OA) and slowly healing damaged tissues are PRP, BM and stem cells. These methods are obtained from patients' own blood or tissues. These therapies have an anti-inflammatory, analgesic, reparative and antidegenerative effect. These thus treated disease states are characterized by inhibition of the inflammatory response, dysfunctional macrophages resulting in inability to fight infections, impaired angiogenesis, fibrous tissue accumulation and accumulation of abnormal extracellular matrix (1).

Platelet-rich Plasma (PRP)

PRP is the autologous concentration of a large number of platelets in a small volume of autologous plasma, which is obtained by centrifugation of autologous blood (2). Platelet concentration provides high levels of a number of bioactive growth factors that stimulate the regeneration of injured tissues and organs (3, 4). PRP also contains fibrin, fibronectin and vitronectin. These proteins are capable of enhancing cell adhesion and acting as a matrix for the
formation of bone, connective tissue and epithelium (2). The activated platelets once in contact with exposed endothelium in wounds or injured tissues, release the growth factors contained within them that stimulate the regeneration of injured tissues. The main growth factors that the platelets contain are platelet growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF) and epidermal growth factor (EGF) (5, 6). Tissue regeneration stage depends on growth factors which involves complex overlapping processes that are categorized as haemostasis, inflammation, proliferation and remodeling. Once tissue damage occurs, a hematoma is formed at the site of the disorder, the platelets adhere to the available collagen, forming a clot. An inflammatory phase begins with platelet activation, resulting in release of growth, bioactive and hemostatic factors (6).

Each factor plays a unique but important integrative role in the early stages of initiation of the clotting cascade. Neutrophils and macrophages access the site of damage within hours, thereby aiding the onset of phagocytosis of necrotizing tissues. The proliferative phase begins within a few days of injury onset. It is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound size reduction. At the end of the recovery period, the remodeling phase takes place; it involves collagen maturation and apoptosis of surplus cells, which may take several weeks to months from the time of injury. The duration of this period depending on the degree of tissue damage. Following the model described above, acceleration of wound healing by applying PRP is based on various platelet-derived growth factors that stimulate the various stages of wound repair (7, 8, 9).

In order to optimize PRP extraction protocols, it is necessary to follow several important parameters, including the centrifugation process for PRP extraction should be sterile and accurately performed to produce platelet separation by red blood cells and their sequestration in high concentrations without any damage or lysis that may cause premature release of growth factors. Another important factor is platelet concentration which should be 300 to 400% greater than that in whole blood in order for PRP to have therapeutic effect (2). Lower PRP concentrations are considered unreliable in regeneration of injured tissues (10). The choice between exogenous pre-activation of platelets or not, and number of applications are also important in choosing a suitable protocol.

Marx et al state that a dual centrifugation technique is required to concentrate autologous blood platelets (11).

**Bone Marrow (BM)**

BM is used as a source of cell therapy as it contains inflammatory cellular progenitors identified as important for the repair of damaged tissues as well as mesenchymal stem cells (MSCs) and other multipotent stem cells (12). MSCs have the potential to restore the dermis by differentiation in a number of cell types such as fibroblasts, chondroblasts, myocytes, etc. (13). These cells also release many growth factors and cytokines which are vital for regeneration of damaged tissues. Other multipotent cells, such as hematopoietic stem cells and vascular progenitors, are also present in BM and are likely to contribute significantly for tissue repair (14). The use of direct BM aspirators for regenerative therapy has a number of advantages. Applying natural, uncultivated, fresh and non-reduced volume autologous BM aspirate containing stem cells is considered to be potentially successful source of proliferative elements for the following reasons:

- prevention of tissue rejection problems,
- heterogeneous BM aspirate composition containing both stem cells and other cells with regenerative potential has synergistic effect, and has been shown to enhance the effect of various cell-based therapies,
- rich source of adipose cells, extracellular matrix and growth factors, all of which coordinate interactions between different cell types.

Materials and methods

PRP is derived from blood samples obtained from venous blood. 30 ml of venous blood produces 3–5 ml of PRP. The amount depends on the baseline platelet count and the method used for PRP production.

Principles of Platelet-rich Plasma Preparation

There are two basic methods used for PRP production: PRP method and the buffy-coat method.

PRP method

The PRP method is performed by two consecutive centrifugations. During the first centrifugation, the red blood cells are removed; during the second centrifugation, the platelets are concentrated; they are dispersed in the smallest volume in the final plasma. The whole blood is produced in syringes containing anticoagulant. The initial centrifugation is carried out at constant acceleration to separate erythrocytes from the remaining volume of white blood cells, resulting in the aspirate dividing into three layers - an upper layer containing mainly thrombi and white blood cells; an intermediate thin layer known as buffy coat and rich in white blood cells; and a lower layer consisting primarily of erythrocytes. To obtain a pure PRP product, the two top layers are transferred to an empty sterile tube. Second centrifugation is performed for the purpose of separating platelet-poor plasma (PPP) from PRP. The upper part of the tube volume is mainly composed of PPP which is removed. The rest of the platelets, about 1/3 (5ml of the plasma), which are located on the bottom of the tube in the form of 'blanket’, are homogenized to create PRP (15, 16).

Buffy-coat method

In the buffy coat method, the whole blood is centrifuged at "high velocity" with subsequent collection of only the middle layer containing mainly white blood cells and platelets. This layer of aspirate contains a high concentration of leukocytes. A very thin layer of buffy coat can be produced from a small volume of whole blood (10 ml).

The resulting whole blood is stored at 20 °C to 24 °C before centrifugation. The blood is centrifuged at a high speed, resulting in three layers: a bottom layer consisting of red blood cells; a middle layer consisting of platelets and white blood cells; and a top layer containing PPP. The top layer is discarded from the tube. The "Buffy-coat" layer is transferred to another sterile tube. The aspirate is re-centrifuged at a low rate to cause white blood cell separation or a leukocyte filtering filter is used. The difficulty consists in separating this thin layer from the underlying erythrocyte layer.

There is no agreement in the field whether the platelets should be activated before or after their application. Some authors activate platelets with thrombin or calcium while others apply platelets without activating them in advance claiming better results (17).
**Principles of obtaining BM**

There are a number of potential areas for obtaining BM, it is most commonly derived from the iliac crest, tibia and calcareum. BM production from the iliac crest provides a high average concentration of MSCs compared to other sites. However, with age, a decrease in the absolute number of MSCs is observed thus possessing decreased distribution capacity.

The patient is anesthetized, after which the area of the iliac crest is prepared aseptically. A 2 cm incision of the skin and subcutaneous tissue of the iliac crest is made. A bone marrow biopsy needle is inserted through the cut of the skin in the anterior aspect of the iliac crest (18). The needle is carefully rotated to reach the medulla of the iliac crest. The needle stylus is pulled out and a 5 ml syringe containing 1 ml heparin 5000 IU/ml is inserted into the medulla of the iliac crest to prevent clotting of the BM aspirate (19). After one minute the BM is aspirated with a 5 ml syringe containing 1 ml heparin 5000 IU/ml. The BM is injected into the injury site.

**Discussion**

PRP is an extremely affordable and easy-to-use cellular therapy that helps in the treatment of chronic wounds, severe burns, chronic joint diseases and traumatic injuries. PRP is an autologous cell therapy containing many bioactive growth factors that are involved in the treatment of wounds and tissue regeneration requiring only mild invasive manipulation. Various protocols for PRP production may lead to different platelet concentrations and thus may have different biological effects (10), (20, 21). For this reason, the number of platelets should be one of the key factors in standardizing studies exploring the regenerative capacity of PRP (22). An important variable, which may have a critical effect on platelet concentration and hence the quality of PRP, are the centrifugation protocols that depend on the rotational speed, and the time and number of the centrifugation process itself (4); (23). Using high RPM can cause premature platelet activation during preparation and thus reduce the regenerative potential of the platelets. Graziani et al. proves in an in vitro study that the biological stimulation of cell growth varies with dose. A comparison of different concentrations indicates that moderate concentrations of 2.5 times produce better in vitro results on proliferation and function of fibroblasts and osteoblasts than concentrations of PRP that are less or more concentrated (24). Rappl et al. note that studies with one to three times of concentrated PRP showed better wound healing results than those with higher concentrations (25). As a regenerative cell therapy, PRP has a wide clinical use: accelerated healing of fractured bones and bone grafts (24), (26, 27), treatment of tendonitis (28), treatment of chronic slow healing wounds; and treatment of joint diseases, etc.

As a first step towards this standardization is the choice of a more efficient and therefore, more accessible method of obtaining PRP to make this type of cell therapy more widely available to a larger number of patients and veterinary medical practices. In general, the technique for obtaining PRP, described in this report, can be applied in the treatment of various clinical cases.

**References**


