

## Review Article

# Overview: Autologous Peripheral Blood Mononuclear Cells

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## Keywords

Autologous Peripheral Blood Mononuclear Cells; Pall Celeris System; Wound healing Macrophages

## Background

The implant of autologous mononuclear cells is an avant-garde method based on the use of a cell population within our body to regenerate tissues that have been damaged by various pathological events. The biological assumption is the richness and complexity of biochemical and cellular phenomena inherent in both organism response to damage and tissue regeneration. The key role is played by the mononuclear phagocyte system which regulates and modulates the activity of mesenchymal stem cells capable of differentiating and providing tissue repair. This system does not only have a traditional "scavenger" function within the immune system, but it is also of central importance in the modulation and activation of the response to tissue damage, be it traumatic, surgical, or degenerative. In this review we summarize the main features of this method and the most common uses in clinical practice where the interest is growing considering both the powerful, rapid and documented regenerative response of the various "target" tissues: vascular, cartilage, bone, muscle and tendon tissue.

## Autologous Peripheral Blood Mononuclear Cells

### Definition

Autologous Peripheral Blood Mononuclear Cells (PBMC) are an autologous cell concentrate (monocytes, macrophages, B-T lymphocytes, Natural Killer cells, CD34<sup>+</sup> hematopoietic stem cells, KDR<sup>+</sup> stem cells, Endothelial Progenitors Cells-EPC) of the latest generation with high angiogenic and regenerative capacity, taken from the patient's peripheral blood [1].

### Mechanism of action

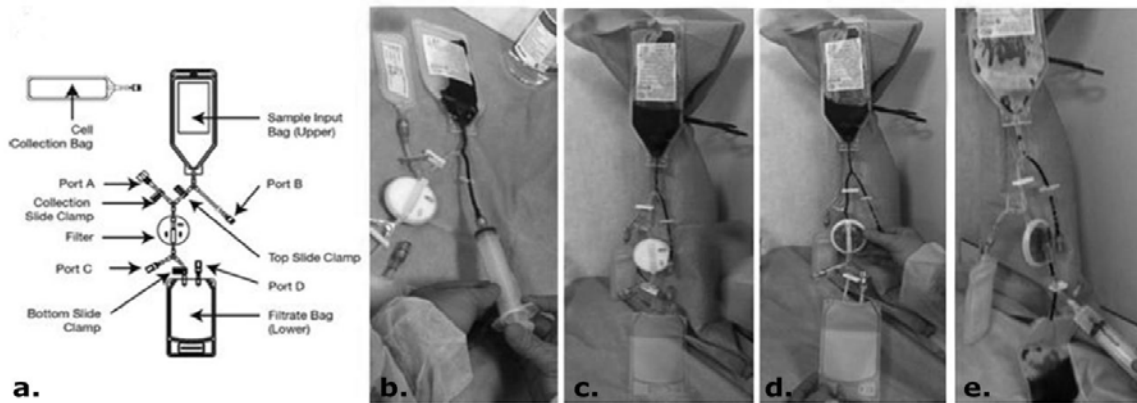
Cell therapy with PBMNC is based on mimicking the physiological response of these cells to tissue and ischemic damage, increasing their speed and intensity of action through implantation into the damaged/ischemic tissue. The angiogenic effect is fundamentally paracrine and consists of the release by monocytes of angiogenic cytokines

(VEGF and bFGF) and growth factors [2,3]. Macrophages (MPs) are critical for tissue repair and regeneration but can also contribute to tissue damage and the genesis of fibrosis. MPs can have a variety of functional phenotypes in response to different stimuli; two of the best characterized *in vitro* phenotypes are a proinflammatory "M1" phenotype, produced by exposure to IFN- $\gamma$  and TNF- $\alpha$ ; and an anti-inflammatory "M2a" phenotype, produced by the presence of IL-4 or IL-13. M2a Mp, often referred to as "wound healing" Mp, as it is involved in tissue repair [4]. MPs show a "classically activated" or M1 proinflammatory phenotype during the early stages of tissue regeneration in response to pathogens, IFN- $\gamma$  and TNF- $\alpha$ , and later an M2a reparative phenotype [5-8]. However, phenotypic markers that appear to be coregulated, in cultures MPs can be expressed independently of each other *in vivo*.

It was initially thought that naïve MPs were activated upon exposure to bacterial components and that IFN- $\gamma$  and TNF- $\alpha$  increased production of inflammatory cytokines and ROS by enhancing their killing ability. In contrast, the main effect of anti-inflammatory cytokines on MPs was assumed to be deactivation, hence resolving the inflammation. However, Stein et al demonstrated that IL-4 is not only a "signal off" for immune activation of MPs as it also increases CD206 mannose receptor activity and enhances endocytosis of mannosylated ligands of murine MPs. Therefore, IL-4-treated MPs were defined as "alternatively" activated compared with "classical" activation by IFN- $\gamma$ /TNF- $\alpha$ , and the concept of general deactivation thought to occur previously with IL-4 treatment was lacking [9].

Other studies have shown that stimuli previously considered MPs "deactivators" produce different phenotypes of macrophage activation *in vitro*. The term "alternative" activation has thus been coined to include these new phenotypes, now referred to as M2a/b/c, whereas "classical" activation is referred to as M1 [10].

The M2a phenotype is produced *in vitro* by exposure to IL-4 or IL-13, which act through the common IL-4Ra receptor with increased expression of CD206, arginase, and TGF- $\beta$  [11-13]. The M2b phenotype is produced by exposure to a combination of IgG and LPS immune complexes, which increases IL-10 production and decreases IL-12 production, conferring potent anti-inflammatory properties [14,15]. *In vitro* exposure to IL-10 or glucocorticoids produces the M2c phenotype, which is characterized by high IL-10 and low IL-12 production [15] as well as the increased expression on the cell surface of the CD163 scavenger receptor [16,17]. The Mp phenotype can also be altered by phagocytosis of apoptotic or necrotic cells [18,19]. Together, these studies have shown that far from being simply activated or deactivated by proinflammatory versus anti-inflammatory cytokines, MPs can adopt a variety of different phenotypes in response to different stimuli.



**Figure 1:** Pall Celeris system. A: Model of the Pall Celeris system; B-E: Images for Pall Celeris system use; B: Blood loading into the sample input bag through port B; C and D: Filtration by gravity. E: Filter backflush to recover Total Nucleated Cells (TNCs) with the use of 14mL of saline solution through port C.

### Product Requirements, Collection Methods and Clinical Use

PBMNCs are autologous blood components; thus, they are collected, prepared, and stored in the same manner as allogeneic blood components; and are identified, stored, transported, and distributed separately from blood components for allogeneic use.

They are blood components for non-transfusion use and can be used according to the following uses established by law:

- Use on skin or mucous surfaces (topical use);
- Intra-tissue or intra-articular infiltration;
- As a material to be applied locally at surgical sites, alone or in combination with non-cellular biological material (e.g., bone bank tissue) or medical devices;
- As material to be used “*in vitro*”, in laboratory procedures, for approved clinical studies according to current regulations.

The production, identification, traceability, appropriateness, informed consent, and consent to the processing of personal data in the context of clinical trials, allocation, delivery, and hemovigilance of blood components to be used for non-transfusion use, the same regulatory provisions apply as for blood components for transfusion use, except as provided in paragraph 7 below [20]. Autologous blood components for non-transfusion use are produced within the Transfusion Services or their organizational units; the production may take place outside the Transfusion Services within the limits and in the manner specified in the SIMTI Recommendations [22,23]. They are obtained from a patient with no risk of bacteremia; biological validation tests must be performed at the beginning of a therapeutic cycle, with a maximum validity period of 30 days; biological validation tests can be omitted if sampling, production, and application are planned in a single session without any preservation of the product [23].

The collection of PBMNCs, to perform a proper cell therapy with high regenerative angiogenic capacity, involves the use of the Pall Celeris System, which is a Point-of-Care (POC) device based on selective filtration, designed to obtain a high concentration of Peripheral Blood Total Nucleated Cells (PB-TNC). The unique

feature of the system is its ability to eliminate the contamination of highly pro-inflammatory granulocytes [21]. Spaltro et al. studied PB-TNCs isolated from healthy donors by characterizing them using flow cytometry and functional tests, aimed at assessing migratory capacity, ability to form capillary structures, endothelial trans-differentiation, and paracrine cytokine secretion. Human PB-TNCs obtained with the Pall Celeris filtration system have been shown to secrete a panel of relevant angiogenic factors and to migrate in response to Vascular Endothelial Growth Factor (VEGF) and stromal-derived factor 1. Human PB-TNCs have induced neovascularization by increasing the number of collagen fibers, capillaries, and arterioles up to 7 days after ischemia in the murine model [22].

Selective filtration allows cell separation based on membrane electrical charge, with minimal contamination of the concentrate by red blood cells and neutrophils. The system also preserves cell membrane integrity and prevents the release of active molecules and premature cell activation. The cell populations eventually present in the concentrate were previously listed [1]. The volume of peripheral blood to be drawn from the patient to obtain PBMNCs cell concentrate varies from 60 to 120 ml with an average of 100ml. All blood samples were collected with ACD-A Anticoagulant and stored at room temperature until use. The developed kit is easy to use. The concentrate of selected mononuclear cells is characterized by high cell viability because the blood is not stressed but filtered; it is extremely liquid and therefore easily injectable and so far, has not presented any adverse reactions. The infusion should normally be performed within 24 hours of blood collection and under sterile conditions. PBMNCs are isolated using the Pall Celeris system (Pall Medical Corporation) according to the manufacturer’s instructions (Figure 1) [22].

To date, the implant of PBMNCs is used for the already mentioned angiogenic and regenerative capabilities, especially in the treatment of patients with critical limb ischemia that cannot be revascularized or that have undergone revascularization [1,24,25] and in orthopedic pathologies such as angular defects of the lower limbs, fractures that have difficulty healing, in tendon or cartilage pathology [25].

Regarding the mode of application, dosage, frequency, and duration of treatment, please refer to the various specialist clinical protocols.

## Conclusions

The implant of autologous peripheral blood mononuclear cells, thus belonging to the patient himself, is an innovative technique, sophisticated but at the same time simple and repeatable; through this procedure, it is possible to accelerate the process of tissue regeneration and facilitate the recovery from trauma, injury, ischemic, degenerative and surgical damage.

## Declaration of Interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

## Authorship Contributions

All Authors contributed equally to the study.

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