Orthobiologics 2024: Definition, Manufacturing, and Mechanism of Action of the Most Commonly Used Alternatives Currently Used in Orthopedics

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ABSTRACT

Orthobiologics is emerging as a new subspecialty of orthopedics, with gradual acceptance. While platelet-rich plasma (PRP) and bone marrow concentrate (BMC) provided the initial catalyst for the widespread use of biological therapies in orthopedics due to their ease of preparation and application, there have been significant advances in the last decade, with numerous clinical evidence emerging on the outcomes of other promising biological therapies such as platelet lysate, adipose-derived stromal vascular fraction cells (SVF), and cell cultures. The following article aims to describe the most widely used biological therapies currently used in orthopedics, with special emphasis on their manufacturing process, composition, and mechanism of action.

Keywords: Orthobiologics; platelet-rich plasma; bone marrow concentrate; cell cultures; adipose-derived mesenchymal cells.
Level of Evidence: V

Ortobiológicos 2024: definición, elaboración y mecanismo de acción de las alternativas más utilizadas hoy en Ortopedia

RESUMEN

La ortobiología está emergiendo como una nueva subespecialidad de la Ortopedia, con una aceptación gradual. Si bien el primer impulso del uso masivo de las terapias biológicas en Ortopedia vino de la mano del plasma rico en plaquetas y el concentrado de médula ósea por su elaboración y aplicación fáciles; en la última década,se han producido avances importantes y ha surgido numerosa evidencia clínica sobre los resultados de otras terapias biológicas prometedoras, como el lisado plaquetario, las células mesenquimales derivadas del tejido adiposo y los cultivos celulares. Este artículo tiene como objetivo describir las terapias biológicas más utilizadas actualmente en Ortopedia, con especial énfasis en su proceso de elaboración, su composición y mecanismo de acción.

Palabras clave: Ortobiológicos; plasma rico en plaquetas; concentrado de médula ósea; cultivos celulares; células mesenquimales derivadas del tejido adiposo.
Nivel de Evidencia: V

INTRODUCTION

The term "Orthobiologics" refers to a type of orthopedic treatment that uses natural substances derived from the body for the healing of musculoskeletal and degenerative injuries. It is essentially a combination of two words: "ortho," relating to Orthopedics, and "biologics," referring to substances derived from the body itself (not to be confused with the pharmaceutical industry's use of the term, which refers to a category of drugs derived from living organisms using recombinant DNA technology). Over the last 20 years, orthobiologics has gradually emerged

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as a new subspecialty of Orthopedics, with a mix of enthusiasm, hope, and some disappointments. Orthobiologics is a clear example of the advancement of translational medicine, where promising laboratory discoveries have been turned into concrete clinical applications. This has led to the publication of an exponential number of clinical trials and meta-analyses in the last decade, covering common musculoskeletal diseases such as osteoarthritis, tendinopathies, and cartilage lesions, among others.[1-5]

While the initial push for the widespread use of biological therapies in orthopedics came from platelet-rich plasma (PRP) and bone marrow concentrate due to their ease of preparation and application, the last decade has seen significant advances. Much clinical evidence has emerged on the outcomes of other promising biological therapies, such as platelet lysate (PL), adipose-derived stromal vascular fraction cells, and cell cultures.

The purpose of this article is to describe the most widely used biological therapies currently employed in Orthopedics, with a particular focus on their manufacturing process, composition, and mechanism of action.

PLATELET-RICH PLASMA

PRP is an autologous biological product obtained from blood through differential centrifugation. It aids in natural tissue regeneration, as it contains growth factors such as fibroblast growth factor type 2, platelet-derived growth factor, tissue growth factor β, vascular endothelial growth factor, and insulin-like growth factor,[6-8] among others, which bind to the plasma membrane of mesenchymal cells to trigger their proliferation and activation.[9] This binding generates positive feedback in the microenvironment, causing more platelet rupture, the release of growth factors, and subsequent binding of the growth factors to the cell membrane. This results in further proliferation and differentiation until the inflammatory response is inhibited and regeneration is achieved.[10] These components not only regulate cell migration and proliferation but also contribute to angiogenesis and tissue remodeling, creating a favorable microenvironment that enhances tissue repair and regeneration.

Growth factors are crucial to this process. They initiate regeneration by inhibiting apoptosis, producing anabolic and anti-inflammatory effects, and activating cell proliferation and differentiation.[11]

Once platelet activation occurs after injury, the factors are secreted and bind to target cells to stimulate cell proliferation, neovascularization, matrix formation, and collagen synthesis.[5,6,12] In the case of bone regeneration, for example, platelet-derived growth factor binds to the plasma membrane of bone cells to stimulate remodeling, mitosis, and phagocytosis of damaged tissue.[13,14] Tissue growth factor β has been shown to regulate proliferation, differentiation, chemotaxis, and adhesion to progenitor cells. It is also a potent inducer of chondrogenesis, positively regulating type II collagen production in mesenchymal stem cells.[15] Both chondrocytes and osteoblasts possess membrane receptors for tissue growth factor β, supporting the theory that this molecule plays a significant role in the process of bone and cartilage regeneration.[16] Vascular endothelial growth factor plays a critical role in angiogenesis and cartilage regeneration.[17,18] Furthermore, it acts synergistically with osteogenic proteins, such as BMO4 and BMO2, aiding in cell recruitment, prolonging survival, stimulating angiogenesis, and accelerating cartilage resorption and bone mineralization.[19,20]

To obtain PRP, a blood sample is needed, which is then concentrated five times through a series of differential centrifugations, from which the phase containing the concentrated platelets is taken (Figure 1). If indicated, the lymphocyte fraction can also be included to assist in the regenerative process. The platelet count and clotting time are determined from the obtained fraction, with the values provided in a certificate of analysis along with the prepared product, ready to be injected into the patient.

The entire process is conducted under type II biosafety laboratory conditions, adhering to all necessary sterilization protocols. This product can be obtained in approximately two hours and serves as a good alternative for treatments that need to be done quickly.

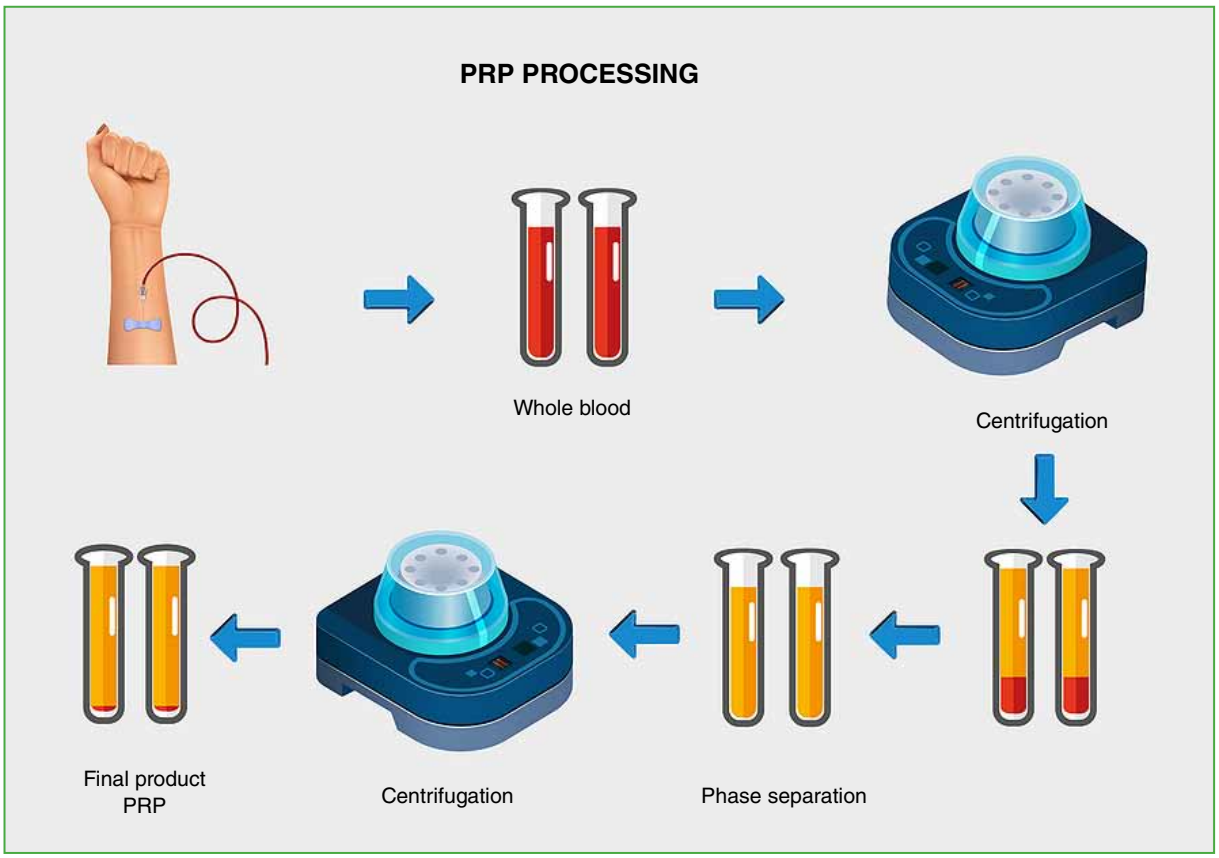

Figure 1. Platelet-rich plasma (PRP) processing. PRP is obtained through a sequence of differential centrifugations from a peripheral blood sample. The final product is applied locally to the area of injury.

PLATELET LYSATE

PL is one of the richest sources of bioactive molecules that can be obtained from a peripheral blood sample. It is considered the evolution of PRP, as both cell proliferation and stem cell differentiation is significantly higher when used in combination. Instead of being a platelet concentrate, it is a concentrate of autologous growth factors obtained from the same individual. In recent years, it has gained increased attention because its preparation is acellular, thus reducing the consequences of immunogenicity while containing high concentrations of growth factors and cytokines. It can be cryopreserved and stored for long periods, unlike PRP, which cannot be exposed to temperatures lower than 4°C, as the platelet mixture is extremely sensitive to temperature.[21,22]

To obtain PL, it is necessary, as in PRP, to have a blood sample collected in the presence of anticoagulants.[23] Similar to PRP, the sample must be sent to the laboratory for processing. PL is obtained through a combination of differential centrifugation interspersed with mechanical or chemical lysis of the platelets, followed by the purification of the obtained factors (Figure 2). This process takes about 8 hours in the laboratory, so it is convenient to perform it overnight.

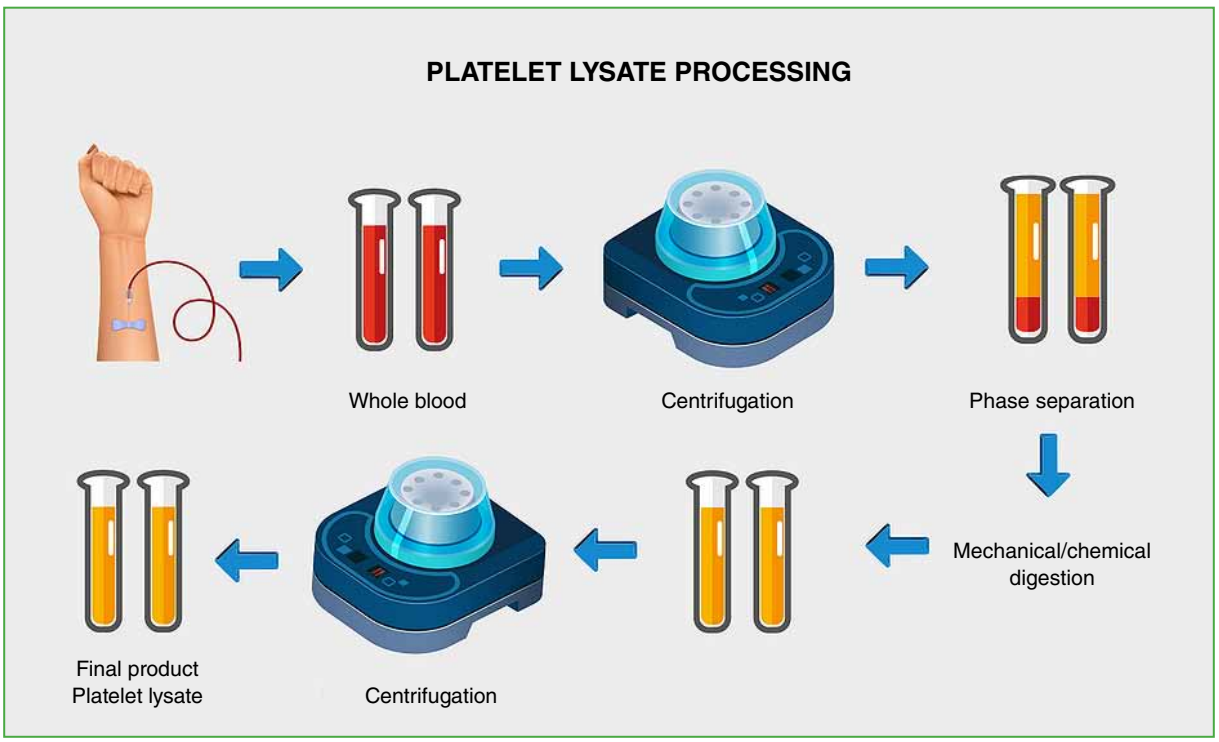

Figure 2. Platelet lysate processing. Platelet lysate is obtained through a sequence of differential centrifugations from a peripheral blood sample. Because it can be stored, a single extraction can be performed for all applications, and the doses to be used can be preserved. Like PRP, the final product is applied locally to the area of injury.

In general, the physician obtains the sample from the patient the day before it is to be used and sends it to the laboratory. Immediately prior to delivery, the product undergoes a final procedure to ensure the quality and potency of the PL. The sample is delivered to the physician's office just a few minutes before the consultation, ready to be injected into the patient, along with a certificate of analysis containing the initial platelet count prior to lysis and clotting time control.

Like PRP, the entire product is manufactured in a Type II biosafety laboratory under the strictest biosafety conditions and controls.

One of the significant advantages of PL over PRP is that it can be stored. This means that if the patient is to receive more than one application, a single blood collection can be made, taking into account the volume necessary for all applications. The professional will be able to schedule the applications in a more orderly manner and will only need to notify the laboratory a few days in advance so that the sample will be available and ready to use just minutes before the medical consultation.

CELL CONCENTRATE

Cell concentrate is a heterogeneous composition of cells, including, among others, endogenous mesenchymal stem cells, and can be used in regenerative medicine. It can reduce apoptosis of surrounding cells, inflammation and fibrosis by activating physiological regenerative mechanisms through cell proliferation and differentiation. In addition, it has the potential to differentiate into multiple lineages, including osteoblasts, adipocytes, myoblasts, and epithelial cells. It can contribute to angiogenesis in a paracrine and autocrine manner[24] and modulates the inflammatory response, collaborating with the recruitment of molecules to the site of injury.[25]

PRP and cell concentrate, whether derived from bone marrow or adipose tissue, have been shown to have synergistic and complementary effects on tissue regeneration.[26,27]

Cell concentrate can be obtained from bone marrow (bone marrow concentrate) or adipose tissue (vascular stromal fraction). In both cases, a concentrate of nucleated cells is obtained, from which a specific and unique population of fibroblasts, endothelial cells, immune cells, hematopoietic cells, pericytes, vascular cells, and mesenchymal cells, among others, is derived. The difference between these tissues lies in the concentration of mesenchymal cells; adipose tissue has a concentration of mesenchymal stem cells of about 3%, while the concentration in bone marrow is significantly lower.

Another consideration when choosing the type of sample to use is the method of tissue collection. In the case of bone marrow, it may be obtained from the iliac crest, sternum, or any other bone of the practitioner's choice, usually in the operating room under general anesthesia. Conversely, adipose tissue is extracted in a doctor's office, without the need for preparation, under local anesthesia at the collection site.

To obtain bone marrow, at least 60 ml of tissue must be processed in a syringe containing heparin. It is then sent to the laboratory for processing, which takes approximately 4 hours. This product is obtained through differential centrifugation (Figure 3). The final product is composed of a concentrate called bone marrow concentrate, composed of nucleated cells such as fibroblasts, immune cells, endothelial cells, and mesenchymal cells. It is delivered to the office or operating room a few minutes before application, with a certificate of analysis indicating the total number of nucleated cells, the percentage of cell viability, the total volume delivered, and the medium in which it is resuspended (physiological solution, PL, PRP, etc.). This product cannot be stored and must be used within 6 hours.

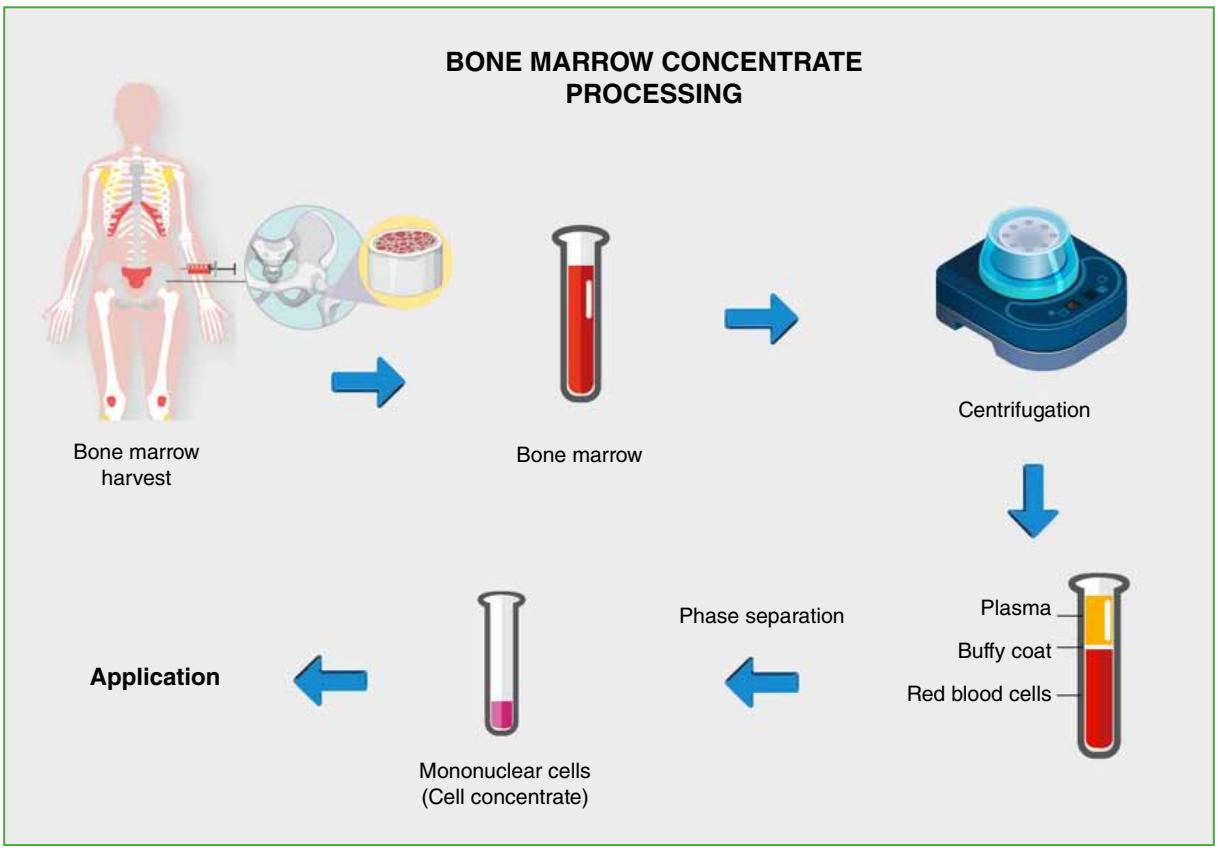

Figure 3. Processing of bone marrow concentrate. Bone marrow concentrate is obtained through centrifugation of a bone marrow sample. The buffy coat is isolated for application and contains a concentrate of nucleated cells, including mesenchymal cells.

For the adipose tissue concentrate (called stromal vascular fraction), at least 1 g of fat should be obtained and taken to the laboratory for processing. The processing time is about 5 hours; for this reason, the sample is usually taken the day before application (Figure 4). The final sample is sent to the office or operating room, ensuring that cell viability and cell number remain stable for up to 6 hours after processing.

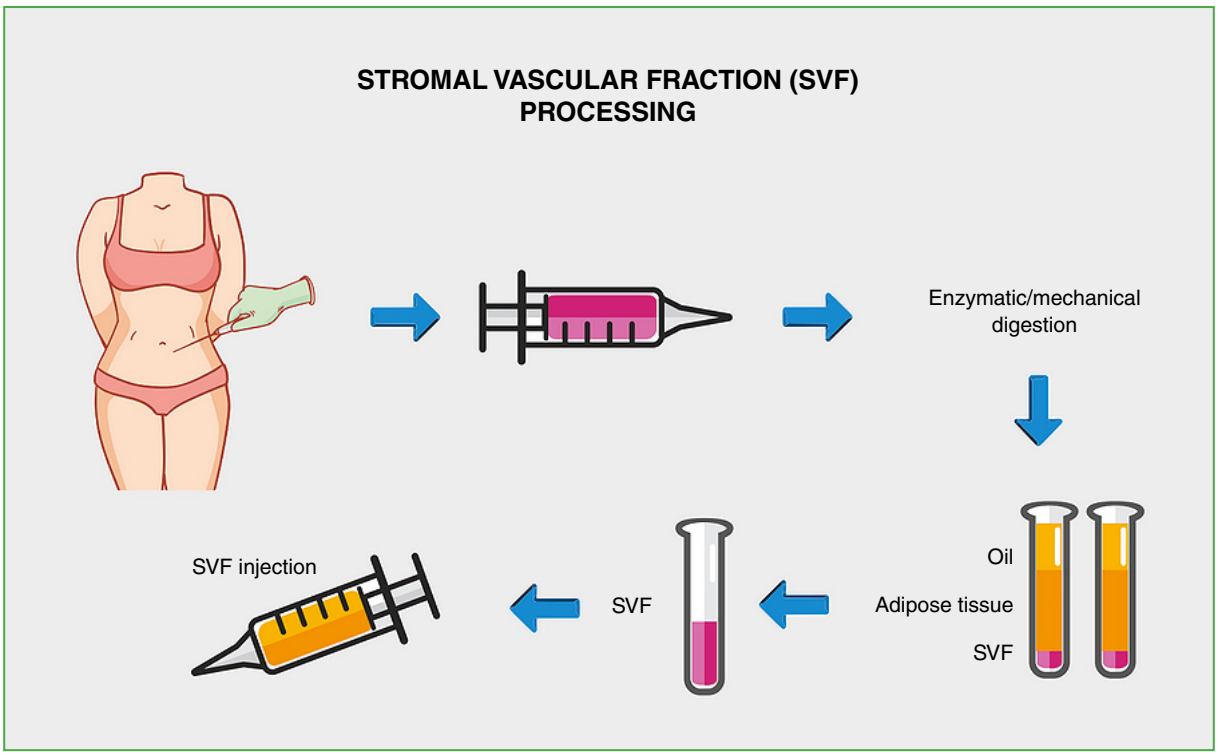

Figure 4. Processing of the stromal vascular fraction from adipose tissue. Adipose tissue is subjected to mechanical and enzymatic digestion. It can be obtained through a small incision to remove a tissue fragment or by liposuction. Like bone marrow concentrate, it contains a population of mononuclear cells, including mesenchymal cells. The final product is applied to the area of the lesion and can be combined with platelet lysate or PRP to enhance its effect.

The rationale for combining PRP with cell concentrates is based on the fact that PRP provides an optimal microenvironment in which cells can trigger cell proliferation and differentiation[2,9,18] and can act as a biomaterial to attract and retain mesenchymal stem cells on-site for longer.[19,20]

CELL CULTURE

Cell culture can be obtained from multiple tissues, such as adipose tissue,[24] using a minimally invasive technique. In this way, mesenchymal stem cells have become important candidates for therapies based on regenerative medicine and tissue engineering.[23] These cells are used for the treatment of various cell types, such as bone,[17,18] cartilage,[19] tendon,[20,21] and muscle.[22,24]

The advantage of cell culture over any of the cell concentrates is that the cell population is 95% pure mesenchymal stem cells, ensuring that all cells will participate in the regenerative process. They are usually combined with PL or PRP to achieve a synergistic effect.

The number of cells to be used varies according to the protocol chosen by the professional, as well as the size of the lesion and the organ involved.

The processing time is approximately one month to reach the required cell number (Figure 5). It has been demonstrated that this biological product is the most effective for tissue regeneration, with effects that are maintained longer than with other methods.[23,26]

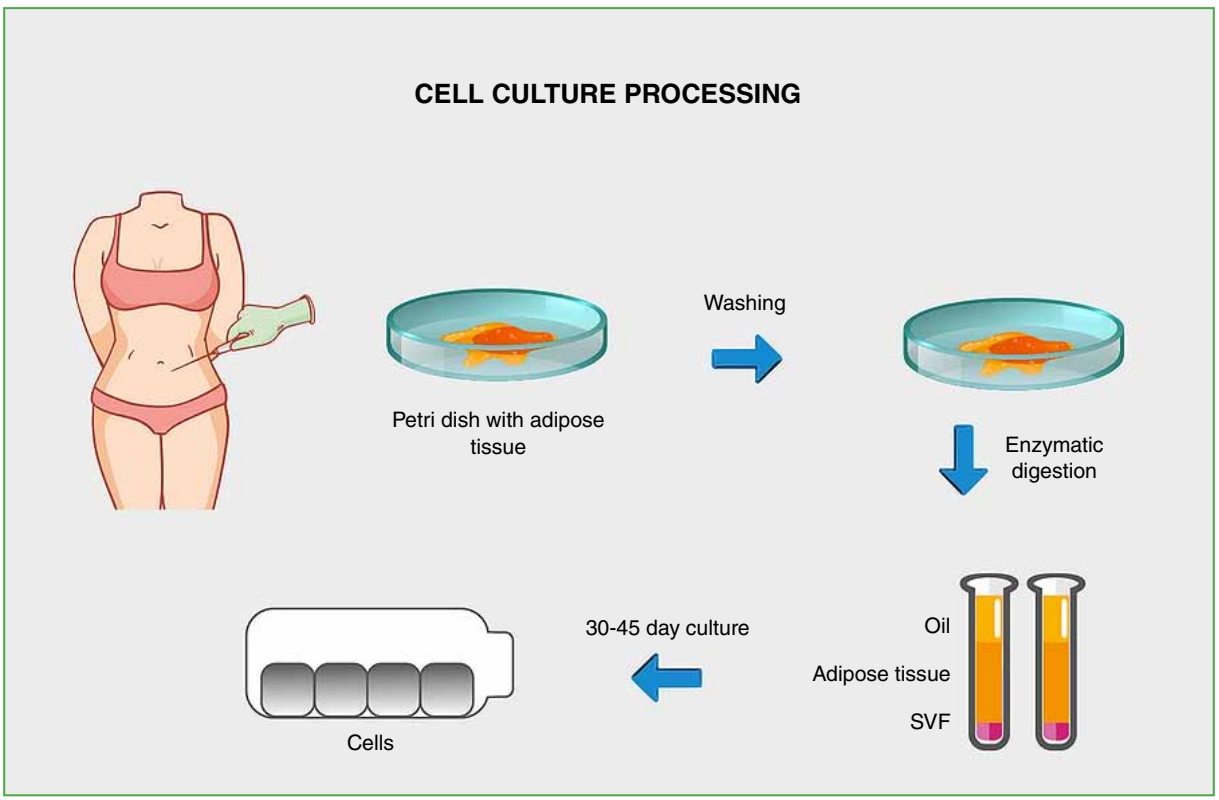

Figure 5. Processing of cell culture from adipose tissue. Once the stromal vascular fraction is obtained, the use of differential media favors the growth of stem cells over other cell types. The cells are cultured until a population composed of 95-98% mesenchymal cells is achieved. Since the product can be cryopreserved, it is possible to process more than one dose to avoid additional removal of adipose tissue.

CONCLUSION AND OUTLOOK

The thought process of the trauma surgeon is clearly shifting from purely mechanically oriented interventions to incorporating and respecting biology. Orthobiologic agents such as PRP, bone marrow-derived connective tissue progenitor cells, adipose tissue, and cell cultures have enormous potential to address deficiencies in soft tissue healing. The main current limitation is the variability in the composition and biological activity of orthobiologic formulations, which makes it difficult to choose the optimal treatment for a specific tissue or disease. Current data suggest that orthobiologics "modify symptoms," but there is little evidence that they can lead to true tissue regeneration ("modify structure"). Current basic science research lines are directed toward a precise understanding of the underlying cellular and molecular mechanisms of tissue degeneration and repair. This understanding will allow for a more targeted therapeutic approach in which we can choose the optimal orthobiologic treatment for specific orthopedic problems. Emerging therapies, such as the use of exosomes and gene therapy approaches, hold great promise as improved methods to both treat symptoms and influence tissue regeneration.

Conflict of interest: Dr. L. Rossi declares no conflicts of interest. Dr. L. Levi is the Scientific Director of Regenerar Laboratory.

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