RESEARCH ARTICLE

ADIPOSE-DERIVED STEM CELLS AND PLATELET-RICH PLASMA: INPUTS FOR REGENERATIVE MEDICINE

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Abstract

Regenerative medicine and tissue engineering have the aim of restoring function due to tissue damage or organ failure. This goal can be achieved either by stimulating endogenous stem cells or by providing exogenous stem cells along with appropriate biomimetic scaffold and growth factors. Among the different sources and types of stem cells, particular attention has been given to adipose tissue-derived mesenchymal stromal cells (ADSCs). The use of ADSCs as source of adult stem cells offers numerous advantages: the collection technique is easier and less invasive than with bone marrow; these stem cells show a high proliferative rate in vitro and are endowed with multi-differentiative capability and tissue repair properties. On the other hand, therapies implying the use of growth factors for tissue regeneration are widely based on platelets: these anucleated cells are a rich source of growth factors and are physiologically involved in hemostasis, wound healing and tissue repair. Platelet-rich plasma (PRP) is an autologous platelet concentrate with antibacterial and anti-inflammatory properties and a vehicle and source of growth factors. Due to these properties, PRP is increasingly used for regenerative medicine purposes, especially in the field of wound healing and osteoarthritis. In this review article, we give an overview of the combined action of ADSCs and PRP in therapeutic applications for these pathological conditions.

Keywords: adipose tissue-derived stem cells, growth factors, platelet-rich plasma, wound healing, osteoarthritis

1. Regenerative Medicine and tissue engineering

Various healthcare programs have been implemented by nations in order to address the increasing medical needs of dealing with chronic disease. The current standards of care are largely based on palliative therapies and the use of pharmaceutical drugs. Whereas, allotransplantation, autologous tissue transfer, and the use of synthetic

materials are presently used for treating many acute and chronic medical conditions, such as organ failure, tissue loss due to trauma, cancer ablation or even congenital structural anomalies for which no adequate treatment is available [1, 2]. However, these therapeutic approaches have limitations and risky side effects, including organ shortages, donor site morbidity, allergic reactions, and immune rejection [3].

Regenerative medicine and tissue engineering interventions aim to treat the root cause of the disease linked to progressive cell destruction and irreversible loss of tissue function with the promise to meet the two most urgent needs of organ transplantation: the identification of a new, potentially inexhaustible source of organs and immunosuppression-free transplantation [4]. In other words, instead of simply mitigating the symptoms as traditional (drugs) therapy approaches do, regenerative medicine aims to repair the underlying pathobiology or restore/replace the native cellular architecture and organ function.

Regenerative medicine is a new and rapidly developing interdisciplinary branch of medicine, typically characterized by a convergence of disciplines such as cell biology, biochemistry, molecular embryology, immunology, advanced materials science, and engineering, such as the 3D bioprinting [5]. The goal of regenerative medicine is to replace or regenerate human cells, tissues, or organs in order to restore or establish normal function. It achieves this by delivering functional cells, supporting scaffolds, growth promoting and signal molecules or DNA encoding these molecules. On the other hand, tissue science and engineering is the use of physical, chemical, biological, and synthetic processes to control and direct the aggregate behaviour of cells, thereby tissue engineering can be considered a subcategory of regenerative medicine [6]. In a broad sense, regenerative medicine encompasses some of the knowledge and practice of tissue science and engineering but also includes self-healing through endogenous recruitment or exogenous delivery of appropriate cells, biomolecules, and supporting structures. The field has already made headway in the synthesis of structural tissues such as skin, cartilage, bone, and bladder [7]. The classic regenerative medicine is to isolate specific cells through a biopsy from a patient, to grow them on a three-dimensional biomimetic scaffold under precisely controlled culture conditions, to deliver the construct to the desired site in the patient's body, and to direct new tissue formation into the scaffold that can be degraded over time [8].

2. Role of Stem Cells in Regenerative Medicine

Within regenerative medicine, stem cells have shown great promise. Tissue engineering and regenerative medicine are undertaking the quest of finding the most suitable type of stem cells that could be employed for therapy, and various types of stem and progenitor cells are in meantime being employed in various clinical trials to replace or regenerate damaged organs [9,10].

Stem cells are believed to be part of the internal repair system of the body, where they replace cells that are lost due to normal turnover or pathological conditions. They are unspecialized cells capable of dividing asymmetrical, thereby continuously renewing themselves and giving rise to specialized cell types [11].

A variety of stem cells can be found during the life time of the human body. Embryonic stem cells (ESCs) can be derived from early (preimplantation) embryos and are pluripotent, meaning that they can differentiate into derivatives of all three germ layers (ecto-, endo- and mesoderm). Due to ethical concerns related to the

destroying of an embryo, immune rejection and tumor formation, induced pluripotent stem cells (iPSCs) were obtained by reprogramming adult somatic cells by gene or protein delivery [10,12]. iPSCs are still under intense scrutiny for giving rise to immune responses after reprogramming them and for generation of malignancies such as teratomas.

Adult stem cells are multipotent and are able to differentiate into a limited number of cell types, often those originating from the same germ layer. A type of adult somatic stem cells is mesenchymal stem cells (MSCs), derived from the mesodermal embryonic tissue. MSCs is the more common term used for stem cells with a self-renewal capacity and multipotent ability, that are precursors of cartilaginous, osseous, adipose and other mesenchymal tissues [13]. Even if the bone marrow (BM) is the most common source, MSCs have been identified in skeletal muscle, pancreas, synovium, skin, blood vessels, adipose tissue, and placenta [14,15]. MSCs isolated from different sources share similar characteristics, although recently it has been recognized that subsets of MSCs with differences in protein and gene expression can be identified in the various tissues [16].

MSCs have generated substantial interest in the medical areas of transplant, regenerative medicine and cancer treatment because of their multi-potency and multi-functionality. Besides the induction of angiogenesis [17], these mesodermal cells are potential modulators of hostile injury microenvironments through their immunomodulatory and anti-inflammatory properties with the result of limiting inflammatory damage to the tissues [18-21]. Among the immunomodulatory activities, MSCs have been described to suppress T- and B-cell responses, to modulate the functions of regulatory T cells, and to inhibit the maturation, activation and antigen presentation of dendritic cells [22]. Anti-inflammatory effects by MSCs are gained through the production of a host of molecules, such as for example tumor necrosis factor (TNF)-stimulated gene-6, interleukin-10, prostaglandin-E2, and other bioactive molecules that act on macrophages [23,24]. Because of their versatility, MSC-based therapies are increasingly brought to the clinics and include stem cell implantation or infusion to treat hematopoietic disease, cardiac conditions, Parkinsosn's disease, respiratory diseases, as well as rheumatology and orthopedic morbidities [25].

3. Adipose Derived Stem Cells: Availability, Isolation and Differentiation Capacity

MSCs in the adipose tissue, termed adipose-derived stem cells (ADSCs), have been shown in large to display same biological capabilities as the BM-MSCs [14]. The advantages of ADSCs over BM-MSCs and other adult stem cell types are that ADSCs are relatively easy to obtain from liposuctions performed in local anesthesia, can be obtained in large numbers, are capable of maintaining their phenotype and plasticity after long term in vitro culture and they comprise a low immunogenicity [26]. Based on this, ADSCs have generated great interest and are perceived as the most preferred cell type for tissue engineering and regenerative medicine [27].

Zuk et al. [28] were the first to investigate whether human adipose could be an alternative source of MSCs. These authors obtained human adipose from liposuction aspirate and used collagenase to release stromal cells from the extracellular matrix by processing the so-called stromal vascular fraction (SVF), containing a variety of different types of cells including ADSCs. The isolated adipose stromal cells were cultured with defined media to induce adipogenic, osteogenic, or chondrogenic

differentiation. It was observed that adipose stromal cells were capable of developing intracellular lipid stores, alkaline phosphatase expression, or proteoglycan expression, markers indicative of adipose, bone, and cartilage tissues, respectively. In order to determine if the isolated adipose stromal cells were indeed stem cells, Zuk et al. [29] examined surface antigen expression and differentiation capacity of clonogenic cultures. Using flow cytometry, the authors observed that the clonogenic cells expressed surface antigens similar to marrow MSCs. Moreover, besides the mesenchymal lineage differentiation, the clonal cells were capable of differentiating into neuron-like cells, as judged by morphology and phenotypic marker expression. ADSCs are also prone to stimulate angiogenesis [30, 31], an essential feature for regenerative purposes. Their neurotrophic and angiogenic properties have been shown to be due to the secretion of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GNDF), vascular endothelial growth factor-A (VEGF-A) and angiopoietin-1 [32]. Indeed, the secretome of ADSCs is complex, ADSCs having the property of secrete proteins involved in angiogenesis, wound healing, tissue regeneration and immunomodulation [33]. Moreover, ADSCs have the capacity to differentiate into Schwann cells [34], pancreatic beta cells [35], and hepatocytes [36-38]. Thus, ADSCs are endowed with properties essential in wound healing and might be an interesting source of stem cells for tissue engineering and regenerative medicine for many medical and surgical applications. For example, Phase I and II clinical trials are evaluating safety and efficacy of ADSCs in the setting of myocardial infarction [39]. Other medical and surgical conditions are being treated with ADSCs, demonstrating ADSC's positive effects in tissue engineering and regenerative medicine [40].

In order to determine if adipose tissue is a comparable source of MSCs to bone marrow, yields and differentiation capacities of cells isolated from each tissue were compared. For example, De Ugarte et al. [41] found no significant differences in the number of culture adherent cells per gram of stromal cells obtained from human marrow or adipose tissue. Yet more than double the average mass of adipose tissue (17 g) could easily be isolated from each patient compared to bone marrow (7 g). The authors also cultured isolated cells in various differentiation media, finding no difference in the number of cells that developed lipid droplets (adipogenic cells), or the alkaline phosphate activity of osteogenic cells. However, when induced to differentiate into cartilage, adipose derived cells stained positive for chondrogenesis while marrow derived cells did not. Using similar methods, several other investigations have compared the ability of marrow and adipose cells to differentiate along these lineages and have demonstrated that cells from either tissue possess an equal capacity to become adipose, bone, and cartilage [42, 43]. Overall, these results may be the outcome of different culture conditions and/or the isolation of different subsets of MSCs, highlighting that the potential of ADSCs to differentiate into either osteoblasts or chondrocytes is controversial at minimum. Carbone et al. [44] have recently demonstrated that only when ADSCs were treated with conditioned medium from cultured chondrocytes and osteocytes, are able to produce glycosaminoglycans and mineralized matrix respectively. These results indicate that ADSCs need growth/morphogenic factor supplementation from the tissue environment to be appropriately differentiated to mesodermic lineages.

Taken together, the above evidence demonstrates that compared to bone marrow, a high number of MSCs capable of multi-

lineage differentiation can be obtained from adipose tissue, and that more cues to their differentiation should be found in in vivo models.

Due to their interesting properties, ADSCs have been found useful for plastic surgery applications, including fat grafts, management of difficult wounds, regeneration of local soft tissue defects, bone reconstruction, recovery from acute tissue ischemia of vascular origin, and scar management [45-47]. The optimal delivery system has to be likely tailored for each morbidity to cure and that is why plenty of methods have been evaluated for ADSC treatment, including systemic administration, local injection, topical applications, and different scaffolds [47]. In this last case, many approaches are being harnessed such as fibrin sealant [48], collagen gel [49], hydrogel [50], dermal substitutes [51-53], as well as cell sheets [54] and composite polymer/ceramic scaffolds along with ADSCs [55].

4. Plated-Rich Plasma in Regenerative Medicine

The current practice of regenerative medicine encompasses not only the use of mesenchymal stem cell therapy but also platelet-rich plasma (PRP), a concentration of blood-derived human platelets in a small volume of plasma. Platelets are non-nuclear cellular fragments derived by megakaryocytes localized in the bone marrow. Mitochondria and the dense tubular system are responsible for providing energy, messengers for reactivity and platelet functions. Platelets are crucial for prevention of blood loss after vessel injury, a process known as hemostasis. They contribute to normal hemostasis in several different ways. First, they adhere to the extracellular matrix of the wounded vessel and prevent blood loss by acting as a physical barrier, and the effectiveness of this mechanism is increased by the ability of platelets to bind to each other in an interaction called aggregation. In addition, platelets contribute to hemostasis by secreting vasoactive substances such as thromboxane A2 that contributes to hemostasis by constricting the wounded vessel [56]. It has also been established that platelets are important for blood coagulation induced by vessel injury with consequent release of tissue factor (TF). Briefly, TF induces a cascade of events where proteases serially cleave each other, which results in the production of a blood clot composed of fibrin. The fibrin clot contributes to the physical blocking of blood loss through the wounded vessel. Platelets affect the process of blood coagulation by acting as an attachment site for coagulation proteases. This facilitates the interactions between coagulation proteases and it also protects the coagulation proteases from degradation by protease inhibitors [57].

Besides hemostasis, platelets are crucial for tissue repair and vascular remodelling [58-60]. They produce cytokines, chemokines and growth factors promoting recruitment, adhesion, and proliferation of adult stem cells. Moreover, platelets provide survival signals to monocytic, endothelial, and neural stem cells [61]. The first stage of normal wound healing, immediately following injury or insult, is inflammation, where activated platelets adhere to the site of injury releasing growth factors (Table 1).

Table I

Platelet-derived growth factors

Growth factor	Function
Transforming Growth Factor (TGF-β)	promotes formation of extracellular matrix and regulates bone cell metabolism
Platelet-Derived Growth Factor (PDGF)	promotes cell replication, angiogenesis, epithelialization and granulation tissue formation
basic Fibroblast Growth Factor (bFGF)	promotes proliferation of endothelial cells and fibroblasts and stimulation of angiogenesis
Epidermal Growth Factor (EGF)	promotes cell differentiation and stimulates re-epithelialization, angiogenesis and collagenase activity
Vascular Endothelial Growth Factor (VEGF)	promotes angiogenesis
Connective Tissue Growth Factor (CTGF)	promotes angiogenesis, vessel permeability, and stimulates mitogenesis for endothelial cells

The theoretical concept that concentrating platelets at the injured site could accelerate and optimize the healing mechanisms set the rationale for the development and continued research into the use of PRP in the clinical application for regenerative medicine [62].

5. Combined Effect of ADSCs and PRP

ADSCs have a great potential for use in tissue repair and regeneration in the field of plastic and reconstructive surgery. ADSCs are isolated by enzymatic digestion, filtration, and centrifugation and are typically expanded in monolayer on standard tissue culture plastic with a basal medium containing 10% fetal bovine serum (FBS) [44]. FBS seems to be essential for cell culture, but it is not safe when the cultured cells are to be used in regenerative medicine. Animal-derived serum may contain xenoproteins that may cause rejection, and may contain transmissible infectious agents [63].

In order to eliminate the use of animal products in human ADSC cultures, platelet-rich plasma (PRP) has been recently proposed as a substitute of FBS, since PRP contains a wide range of growth factors, proteins, and enzymes supporting attachment, growth, and proliferation of cells [64,65].

The advantages of using platelet concentrates in the clinical setting are many. When used as medium supplement, PRP has been shown to promote the growth of ADSCs and maintain their differentiation potential [65]. Furthermore, the antimicrobial and anti-inflammatory

properties of PRP might represent a valuable adjunct to the enhancement of tissue regeneration. Wound healing and neovascularization in a porcine model were enhanced only when ADSCs were topically administered along with PRP [66], and these effects were largely attributed to the large amount of growth factors found in PRP.

The regenerative potential of platelet concentrates and ADSCs on hard and soft tissues has been explored considerably during the last decade. Preclinical studies in animal models have confirmed the synergistic effects of ADSCs and PRP in the healing process of wounds [67] and in osteoarthritis [68,69].

A study by Cervelli and colleagues [70] reported the combination of PRP, autologous ADSCs and hyaluronic acid as a dressing in order to regenerate tissue and achieve epithelialization of wounds localized in the lower limbs, with a significant healing-time reduction in most of the 30 treated patients. Furthermore, the authors reported a fewer number of medications and subsequent improvement of the quality of life.

Pak's group injected ADSCs and PRP in 91 patients with various orthopedic pathological conditions [71]. The follow-up conducted for up to 30 months showed that the treatment was safe, as tumors did not appear at the injection sites. In a study conducted by Bui et al. [72], 21 patients with osteoarthritis from cartilage injury at grade II to III have been enrolled. The goal of this group was to demonstrate the clinical safety and efficacy of autologous transplantation of ADSCs in combination with PRP. They obtained completely reduction of pain levels in 100% of patients without complications, such as tumor formation or microorganism infections.

More recently, the effect of a single injection of autologous ADSCs (as SVF) in combination with PRP administered intra-articularly in ten patients with symptomatic primary osteoarthritis of the knee have been studied [73]. The results demonstrated that, after 2 years from the treatment, ADSCs and PRP could reduce the pain levels in these patients and that the procedure was very safe because it did not caused any complications.

Overall, these studies highlight the great potential of autologous ADSCs combined with PRP as a therapeutic agent in regenerative medicine especially in orthopedic conditions.

6. Conclusions

The discovery of adult multipotent stem cells within the stroma of adipose tissue opened the door to a wide range of therapeutic avenues in regenerative medicine and tissue engineering. Growing autologous tissue from the patient's own stem cells in order to repair damaged tissue and restore tissue function is becoming a reality. A clear advantage of using ADSCs in regenerative medicine and tissue engineering resides in the possibility to harvest a great amount of subcutaneous adipose tissue. Currently, the use of autologous additives such as PRP is a promising approach in enhancing the applications of ADSCs. Preliminary results support the clinical application of PRP for ADSCs-based therapy of osteoarthritis, soft-tissue engineering and wound healing.

To date, a standard expansion method of ADSCs has not been yet established and new methodologies accelerating proliferation of ADSCs while preserving their multipotent differentiation capacities are needed in order to proceed to clinical applications.

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