# Regenerative Strategies in Skin Wounds

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The number of clinical trials evaluating adipose-derived mesenchymal stem cells (AD-MSCs), platelet-rich plasma (PRP), and biomaterials efficacy in regenerative plastic surgery has exponentially increased during the last ten years. AD-MSCs are easily accessible from various fat depots and show intrinsic plasticity in giving rise to cell types involved in wound healing and angiogenesis. AD-MSCs have been used in the treatment of soft tissue defects and chronic wounds, employed in conjunction with a fat grafting technique or with dermal substitute scaffolds and platelet-rich plasma. In this systematic review, an overview of the current knowledge on this field has been provided. Currently, only 72 articles, strictly regarding the use of AD-MSCs, PRP, and biomaterials in chronic skin wounds and soft tissue defects, have been published. The information analyzed highlights the safety and efficacy of AD-MSCs, PRP, and biomaterials on soft tissue defects and chronic wounds, without major side effects.

Keywords: adipose-derived mesenchymal stem cells (AD-MSCs) ; platelet-rich plasma (PRP) ; biomaterials

## 1. Introduction

A scientific, clinical need exists for the development of biotechnologies to improve wound healing (WH), soft tissue defects (STDs), and skin repair (SR) in regenerative plastic surgery (RPS).

The number of investigations evaluating the efficacy of autologous platelet-rich plasma (PRP), adult stem cell-based therapy (A-SC-BT), in particular those based on adipose-derived mesenchymal stem cells (AD-MSCs) and biomaterials, have exponentially increased during the last decade (2010–2020).

As the largest organ of the body, the skin (consisting of the epidermis, the dermis, and its appendices), acts as an important barrier against the invasion of foreign microorganisms and has the functions of immunity, thermoregulation, and metabolic activities [1]. Skin defects resulting from burns, chronic diseases, trauma, tumor resection, and so forth often cause water-electrolyte imbalances and microbial invasion, threatening people’s lives.

Autografts, particularly split-thickness skin graft (STSG) or skin flap transplantation, are considered to be important managements for aiding full-thickness skin defects [2][3]. Due to the disadvantages (e.g., complicated operation, severe damage to the donor area, bloated appearance, high failure rate, etc.), skin flap transplantation is not as widely used in clinical practice as skin grafts [4]. However, despite the better take-in in the early stage, skin grafts lacking sufficient dermal matrix are often hindered by uncontrollable scar hyperplasia, lower mechanical resistance, and so forth in the later phase, leading to graft failure or severe scar formation, which seriously affects the local appearance and functions [5]. Understanding how to avoid severe scar hyperplasia and contracture in the later phase is a key difficulty that needs to be overcome in skin grafting. Whether the skin graft has sufficient blood supply is the main factor affecting the quality of the skin graft [6].

PRP is a high-concentration platelet-oriented plasma obtained from animal or human whole blood via centrifugation [7], which is typically 3- to 7-fold of the mean platelet concentration in whole blood. Containing α-granules, platelets secrete several growth factors (GFs) after being activated, such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and so forth [8][9]. These GFs and other proteins (such as adhesion molecules and chemokines) interact with the local environment to promote cell differentiation and proliferation, which are responsible for re-epithelization and angiogenesis via mesenchymal cell recruitment and extracellular matrix synthesis [8][10]. Nevertheless, PRP is limited due to burst release and its short half-life; that is, 95% of these GFs are secreted within an hour, quickly dilute, and decay into the tissue fluid [11][12]. In this case, although secreted GFs promote angiogenesis and fibroblast maturation, this release process will inevitably cause extensive waste of GFs. In practice, knowing how to avoid the burst release and reduce the waste is a hot issue that needs to be addressed during the use process.

In the past few decades, numerous bi-layer dermal substitutes have been developed and applied for the management of full-thickness skin defects, such as Alloderm® [13][14], Integra® [15][16], Pelnac® [17], and Lando®, of which the low-layer porous collagen sponge scaffold (CSS) function as a dermal regenerate template (DRT). Being the main protein of the extracellular matrix, collagen has excellent biocompatibility with low immunogenicity [18]. Moreover, collagen scaffolds have attracted the most attention due to their sustained drug release properties [19]. Previous studies have demonstrated that the CSSs have surfaces of high porous structure that can be functionalized to provide a biomimetic three-dimensional microenvironment, possessing the ability to host drug molecules to sustain drug release [20]. On the other hand, the porous structure guides inward proliferation and the migration of fibroblasts and endothelial cells, which promotes granulation tissue and angiogenesis for wound healing with less scarring [21][22]. However, the second-step skin graft surgery is usually performed two to three weeks after CSS implantation [23], which increases the patient's pain and prolongs the patient's hospital stay. One-step skin grafting requires the dermal substitute and a split-thickness skin graft to be covered on the wound at the same time. The existence of a non-vascular active dermal substitute may hinder the survival of the graft [24]. It is known that functional surface coatings mediate the cell-surface interaction between the tissues and the biomaterials, which provide alternative strategies for the improvement of bioactivities [25][26].

In this field, the aim of regenerative strategies must be the development of new autologous biotechnologies to promote WH by ex vivo and in vitro culture, or by in vivo regeneration and bio-stimulation. Autologous A-SC-BT has been of great interest for application in WH and SR. Some early efforts in the field focused on isolating primary cells from a biopsy of the tissue of interest and growing the cells ex vivo for subsequent introduction back into the patient.

The preliminary outcomes related to the use of a new regenerative technique to provide autologous A-SC-BT involving human AD-MSCs to be used in patients affected by STDs and chronic skin wounds (CSW) have been reported [27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51]. The AD-MSCs were obtained by multiple procedures of centrifugation, filtration, and/or fragmentation [51]. However, a major limitation encountered in this area has been the difficulty in expanding cells to sufficient numbers for human use, the necessity to perform this expansion in good manufacturing practices (GMP) laboratories, and the viability of the expanded cells [51]. For this reason, the clinical use of A-SC-BT to improve WH and STDs has not been adequately considered.

Alternatively, the use of autologous platelet-derived growth factors, contained in PRP, may represent a valid regenerative strategy for their capacity to promote cell proliferation, differentiation, and neo-angiogenesis, favoring, in vivo, the WH process [52], and hair regrowth (HR) has been reported recently [53][54][55][56][57][58].

In this systematic review, data from investigations reporting the use of AD-MSCs, PRP, and biomaterials in CSW and STD treatments to evaluate such interventions' efficacy, were analyzed.

## 2. Discussion

The use of autologous fat grafting and related AD-MSCs have become popular surgical procedures during the past 10 years, in the treatment of STDs, CSW, scars, and chronic ulcers [27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51].

As in every surgical procedure, the success of fat grafting is dependent upon many factors: the techniques and instruments used to harvest the fat tissue, the fat tissue processing, the volumes of fat implantation, the recipient sites to be implanted, the levels of placement, and even the individual patient. Because of this variability and perhaps because of other factors that are not yet understood, the results of fat grafting with some techniques, in some patients, and in some areas, can be unpredictable. A standard procedure has not been adopted by all practitioners. There is no agreement as to the best way of processing the fat to ensure the maximal take and viability of the graft [27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51]. Successful, three-dimensional sculpting requires meticulous planning and optimizing the harvesting, storage, and transplantation of adipose tissue. There is an unpredictable degree of resorption of the transplanted fat and repeated treatment sessions are usually needed in order to achieve the final result. The average tissue loss because of reabsorption after injection in the buttock varies between 24% and 84% [27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51]. The concept of refining techniques for liposuction and fat injection, according to individual anatomical zones, is essential to the evolution of the procedure [27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51].

Recently, an increasing number of papers have been reported on the use of fat grafting enhanced with stromal vascular fraction cells (SVFs), including breast reconstruction [28][29][30][32][33][35], scars [37][38][39][40][44], ulcers [31][41][47][49], and breast augmentation [51].

AD-MSCs may be identified in the mixed cell population, referred to as SVFs [37][38][39][40][47][49][51]. SVFs containing AD-MSCs might increase fat graft maintenance by improving vascularity and via the secretion of GFs that increase fat survival with the final aim of improving STV.

Several investigators have published articles using fat grafting enhanced with SVFs and AD-MSCs, called by different commercial names, with favorable results employing different procedures of cell isolation [28][29][30][32][33][35][37][38][39][40][41][47][49][51]. Additionally, several studies have been published on the use of centrifuged fat grafting enriched with PRP in Regenerative Plastic Surgery (RPS) [31][32][33][37][38][40][44][45] as RS, compared with traditional procedures [46].

Full-thickness skin defects caused by severe trauma, extensive burns, diabetes, and other reasons are common clinical disorders; their healing is a dynamic and multi-stage process that includes inflammation, angiogenesis, matrix deposition, and cell recruitment [59][60]. Additionally, there are multiple kinds of cells involved, including keratinocytes, endothelial cells, fibroblasts, inflammatory cells, and so forth. [61]. Generally speaking, the conventional treatment is to debride the wound and then perform skin grafting or skin flap transplantation after the wound granulation tissue grows well [2]. Compared with skin flap transplantation, skin grafts have the distinctive advantages of less damage to the donor site and easy operation [4]. However, due to the lack of a sufficient dermal matrix, skin grafts are easily hindered by uncontrollable scar hyperplasia, lower mechanical resistance, and so forth in the later phase [62]. The development and application of skin tissue engineering may improve skin graft treatment to a certain extent. PRP has been reported to be a stocking source for various GFs (e.g., PDGF, IGF-1, VEGF, FGF-2, etc.), which stimulate neo-angiogenetic vascularization and various activities of fibroblasts [63]. VEGF is accepted as the principal stimulatory factor of angiogenesis. PDGF, capable of enlarging blood vessels and forming mature vessels, is a powerful chemoattractant for fibroblasts and smooth muscle cells. Nevertheless, the application of PRP is currently limited by the short half-life period and the burst release (causing low concentration of GFs in situ) [12]. It is necessary to find solutions to improve the clinical application of PRP.

Artificial dermis is a commercially available bi-layer scaffold (including the silicone layer and the collagen-based layer) that has been widely applied in various full-thickness skin defects. The porous structure and suitable pore size of the lower layer (that is, the collagen sponge scaffold (CSS), termed as a dermal regenerate template (DRT)) promote cell adhesion and diffusion, and the connection of pores plays a key role in nutrient transportation and vascularization, significantly reducing contracture and scar formation [64]. Currently, a two-step surgical procedure is the universal criteria in the application of artificial dermis. Briefly, wounds are implanted with bi-layer artificial dermis, following thorough wound debridement in the first stage, and after two to three weeks of the DRT's vascularization, skin grafts are performed to cover the wound bed in the second stage [65][66]. Undoubtedly, the need for two surgical procedures is not only indeed frustrating and inconvenient, but also increases the hospital stay and the risk of infection [67]. Therefore, one-step transplantation (i.e., DRT implantation and skin transplantation are performed in one surgical procedure) implies an attractive clinical prospect. However, the current DRT of artificial dermis commercially available by means of the one-step strategy will counter-intentionally form a barrier between the wound bed and the grafted skin, leading to graft failure [68].

Previous studies have suggested that PRP has positive effects on the implantation of artificial dermis. Formigli et al. [10] reported that artificial dermis combined with PRP demonstrated an improved overall restoration of tissue functions. On the other hand, Harrison et al. [69] explored the use of collagen as a platelet activator in PRP, proving the sustained release effect for growth factors from PRP with collagen. The main challenge of this strategy was understanding how to promote the cells' adhesion, and proliferation, and vascularization into the scaffolds in the short-term after surgery, thus achieving sustained and efficient tissue regeneration with enough vascularization to enable the skin grafts with desired survival.

## 3. Concluding Remarks

In conclusion, this systematic review showed the efficacy of PRP, AD-MSCs, and biomaterials in the therapy of CSW, STDs, and skin defects. Given the current treatments differing in methodology and treatment technique, further studies are needed to define standardized protocols, and large-scale randomized trials still need to be conducted to confirm their efficacy. For these reasons, the authors invite all the audience to improve the level of publications in this field by focusing prevalently on EBM Level 1 studies.

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