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Therapeutic actuality

State of the art. Autologous fat graft and adipose tissue-derived stromal vascular fraction injection for hand therapy in systemic sclerosis patients

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ABSTRACT

Systemic sclerosis is an autoimmune disease characterized by sclerosis (hardening) of the skin and deep viscera associated with microvascular functional and structural alteration, which leads to chronic ischemia. In the hands of patients, ischemic and fibrotic damages lead to both pain and functional impairment. Hand disability creates a large burden in professional and daily activities, with social and psychological consequences. Currently, the proposed therapeutic options for hands rely mainly on hygienic measures, vasodilatator drugs and physiotherapy, but have many constraints and limited effects. Developing an innovative therapeutic approach is crucial to reduce symptoms and improve the quality of life. The discovery of adult stem cells from adipose tissue has increased the interest to use adipose tissue in plastic and regenerative surgery. Prepared as freshly isolated cells for immediate autologous transplantation, adipose tissue-derived stem cell therapy has emerged as a therapeutic alternative for the regeneration and repair of damaged tissues. We aim to update literature in the interest of autologous fat graft or adipose derived from stromal vascular fraction cell-based therapy for the hands of patients who suffer from systemic sclerosis.

1. Introduction

Systemic sclerosis (SSc) is a rare and systemic autoimmune disease characterized by microvascular damages and fibrosis. Life-threatening organ lesions only affect a minority of patients: in contrast, lesions of the hands are almost always present and hand-related symptoms create a large burden in daily and professional activities leading to a significant downgrade in quality of life of the patient, and also psychological distress [1,2]. It has been estimated that hand disabilities account for 75% of overall disability of the disease [3]. Hand disability mainly results from Raynaud's phenomenon (RP), acrocyanosis, ischemic digital ulcers (DU), and from skin fibrosis, which can lead to progressive fingers retraction and spontaneous or traumatic ulcers on the bony prominences in some patients. Patients may also suffer from a non-deforming inflammatory joint disease, acro-osteolysis and subcutaneous calcinosis.

Besides cold/traumatisms protection, moisturizing and regular physiotherapy, specific therapeutic approaches in hand disability in SSc are limited. They are mainly based on vasodilators currently recommended for RP and ischemic DU therapy: calcium channel blockers, phosphodiesterase 5 inhibitor, intravenous prostacyclin and endothelin-1 receptor antagonist. Unlike other autoimmune diseases, immunosuppressive drugs (low dose steroids, methotrexate, cyclophosphamide and mycophenolate mofetil) have a limited efficiency and are mainly proposed in progressive and severe form of the disease.

Autologous hematopoietic stem cell transplantation (HSCT) was recently proven to improve severe and progressive early diffuse cutaneous SSc [4]. Patients in the HCST group experienced

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higher events and mortality in the first year but they had better long-term event-free survival and overall survival, compared to those treated with cyclophosphamide. Indeed, in some cases, this procedure can cause a regression of skin sclerosis assessed by the global modified Rodnan Skin Score (mRSS) [5]. However, HSCT should be reserved for selected patients with a progressive and severe disease. Five cases of transplantation of mesenchymal stromal cells (MSC) from allogeneic related donors have also recently been reported in a severe progressive form of the disease [6]. While there was a therapeutic healing of skin ulcers, due to the heterogeneity of these cases, there is not enough evidence to prove the efficacy of the MSC transplantation.

Autologous fat grafting, also known as lipofilling, is one of the most common procedures in the area of plastic surgery used to restore the defect of soft tissue. Current use of adipose tissue relies not only on its volumizing effect, but also on its regenerative/reparative effect, as grafted fat is much more currently considered than a long-term filling [7]. Trophic properties are not attributed to the mature adipocytes, but rather to the undifferentiated cells. Indeed, in 2001, researchers at the University of California (USA) described the isolation of a new population of adult stem cells from liposuctioned adipose tissue, which share many key properties of mesenchymal stem cells [8]. Although various terminologies were used in literature, these cells are now identified as adipose-derived stromal/stem cells (ASC) according to the IFATS (International Federation of Adipose Therapeutics and Science) and ISCT (International Society for Cellular Therapy) recommendations [9,10]. They are contained within the adipose tissue-derived stromal vascular fraction (ADSVF), which is a heterogeneous mix of cells released by enzymatic digestion of harvested adipose tissue. ASC have multilineage differentiation capacities and provide various paracrine factors involved in immunomodulation, angiogenesis and tissue healing [8,11]. Their frequency is substantially greater in adipose tissue than in bone marrow [12,13]. ASC are becoming well known in the fields of stem cell research and regenerative medicine. However, ASC isolation requires an in vitro selection and expansion process after plating of ADSVF, and thus cannot be immediately used in clinical practice. Contrary to ASC, ADSVF constitutes a minimally processed cell population for immediate use [8]. In addition, ADSVF does not only include ASC, but also a substantial number of other cell types with therapeutic potential, such as vascular endothelial cells and progenitors, immune cells, pericytes and tissue macrophages [14].

For all these reasons, ADSVF cell therapy has emerged as a therapeutic alternative for the regeneration and repair of damaged tissues. The abundant supply of fat tissue, the ease of isolation, the ability to secrete pro-angiogenic growth factors and the abundance of stem/progenitor cells make adipose-based therapy ideal for ischemic and non-healing wounds. In SSc, adipose-derived cell therapy seems to be an attractive source and worthy of attention from clinical translation. Here, we aim to update bibliographic data on the use of autologous fat graft or ADSVF cell-based therapy for the hand of patients suffering from SSc.

2. Composition and biological properties of adipose tissue-derived stromal vascular fraction cells (ADSVF)

At a cellular level, adipose tissue consists of mature adipocytes surrounded by non-adipocytic cells, which include the ADSVF and perform a number of important functions for adipose tissue homeostasis. The dominant cell types in the ADSVF are stromal cells and blood cells. Also, endothelial cells/progenitors and pericytes play a minor part. Harvesting site, lipoaspiration and reinjection techniques are known to influence the quality of ADSVF cells and should be taken into account when developing an adipose tissue cell-based therapy [15–17].

2.1. Composition of adipose tissue-derived stromal vascular fraction (ADSVF)

After fat harvesting by liposuction, ADSVF isolation is achieved through washing, enzymatic digestion and low speed centrifugation, which allow the separation of the floating adipocytes-containing fraction from the denser ADSVF pellet. Processing of lipoaspirate can be performed manually or by using automated devices, which generate ADSVF in a highly quality controlled and consistent manner. Immediate availability of cells allows the injection during the same procedure. The ADSVF contains red cells, platelets, and a heterogeneous population of nucleated cells. According to IFATS and ISCT, distribution of nucleated cells of ADSVF is as follows [10]:

- 25 to 45% of hematopoietic cells;
- 15 to 30% of stromal/stem cells;
- 10 to 20% of endothelial cells and progenitors;
- to 5% of pericytes.

In a previous work, ADSVF cells from 12 SSc patients enrolled in a phase I clinical trial called SCLERADEC were analyzed by flow cytometry [18]. Data on the proportion of the different nucleated cell subpopulations was not significantly different compared to healthy donors (Table 1). Moreover, the frequency of stromal progenitor cells, evaluated using the standard fibroblastoid colony-forming unit (CFU-F) assay, ranged from 1.6% to 8.1% and was similar within ADSVF from SSc patients and controls (F. Sabatier, Cell Therapy Unit results). This data suggests that SSc pathological context does not critically impact the ADSVF quantitative composition. Nevertheless, several reports documented functional alterations of bone marrow derived endothelial or mesenchymal precursors in SSc patients [19,20]. Thus, further studies should address whether the regenerative potential of autologous ASDVF is modified in the context of scleroderma disease.

2.2. Biological properties of ADSVF

It is argued that therapeutic efficacy of ADSVF relies on angiogenic, anti-inflammatory, immunomodulatory and regenerative effects, and is due to the synergic interaction between the

Table 1
Proportion of different nucleated cell sub populations within the ADSVF. Results of flow cytometry analysis performed on the ADSVF isolated from SSc patients and healthy donors.

Cell types	Membrane markers	SSc patients $n = 12$ mean ± SD[a]	Healthy $n = 14$ mean ± SD[a]
Hematopoietic cells	CD45+ CD34–	49.1 ± 18	37.3 ± 10.5
Stromal cells	CD45– CD34brightCD146-CD90+	36 ± 14.5	42.4 ± 12.6
Endothelial cells/pericytes	CD45– CD34dimCD146bright	6.4 ± 6.8	6.2 ± 5.3
Endothelial progenitors	CD45– CD34brightCD146dim	3.4 ± 3.2	6.2 ± 3
Resident macrophages	CD45+ CD14+ CD34dim	5.1 ± 2.3	7.9 ± 5.3

[a] Results are expressed in percent.