1. SCLERODERMA

A. Clinical Response: Clinical response demonstrates a decrease in the progression of the disease and evidence of an improved immune system. In addition to physical examinations prior to stem cell treatment and post-procedure, additional tests to monitor Scleroderma include:
- Skin Score
- Blood Pressure testing
- Pulmonary function testing
- Endoscopy
- Dental testing
- Muscle Enzyme monitoring
- Vascular tests

B. Objective: To provide the patient with a treatment that stimulates cell regeneration and loosens the skin. The Ad-SVF containing adult stem cell procedure should serve to compliment the patient’s current treatment regimen or to promote healing when current treatment is not responding.

2. PRELIMINARIES

A. Background: Scleroderma, also known as systemic sclerosis, is a chronic connective tissue disease that involves the hardening and tightening of the skin and/or connective tissues and it is classified as an autoimmune disease. In some people, scleroderma only affects the skin, however there are other cases where this disease affects blood vessels, internal organs, and the digestive tract [1]. It is estimated that fewer than 500,000 people in the United States are affected by Scleroderma and one third of those people have the systemic form. Localized Scleroderma is more common in children, whereas systemic scleroderma is more common in adults; 4 out of 5 patients are female. Other factors, besides gender, may influence the risk of getting scleroderma such as the ethnic background, race, and age. Scleroderma is more common and more severe in African Americans and Native Americans between ages of 30 to 50 [2], however the exact cause or causes are still unknown.

There are two major classifications of scleroderma, localized scleroderma and systemic sclerosis (SSc), and each have two subtypes:

B. Types of Scleroderma:
- Localized Scleroderma: It is relative mild and usually found on the skin or muscles, and it rarely spreads elsewhere. The internal organs are not affected and people with localized scleroderma, usually do not develop systemic scleroderma [2].
  - Morphea: It is a form of localized scleroderma which is characterized by waxy patches on the skin varying in size, shape, and color. These patched may enlarge or shrink, and often disappear without treatment. It usually appears between ages of 20 to 50, however it is most often seen in young children.
  - Linear scleroderma: It is a subtype of localized scleroderma which frequently starts as a line of hardened waxy skin on the arm, leg, or forehead. Linear scleroderma involves deeper layers of the skin as well as the surface layer, affecting the motion of the joints. Linear scleroderma usually develops in childhood and may affect the growth of involved limbs.

- Systemic Scleroderma (systemic sclerosis): This is a more severe form of scleroderma, affecting connective tissue. It can involve the skin, esophagus, gastrointestinal tract, lungs, kidneys, heart, amongst other internal organs, as well as blood vessels, muscles, and joints [2].
  - Diffuse Scleroderma: This is a pattern that systemic scleroderma may take. Diffuse scleroderma is where the thickening of the skin spreads rapidly and has a higher risk in hardening the internal organs.
  - Limited Scleroderma: It has less risks that diffuse scleroderma. The thickening of the skin spreads slowly and it is usually, but not limited to the thickening of the fingers, hands, and face. The internal organs may be affected, however it happens less frequently and it is less severe than diffuse scleroderma. Pulmonary hypertension is a common condition that can be developed due to limited and diffuse scleroderma.

C. Causes of Scleroderma: The cells in a person with scleroderma lose the ability to stop the production of collagen, therefore creating too much collagen. The over production of collagen thickens the tissues preventing normal functionality. The cause of this event is still unknown and a cure does not exist, however treatments are available to alleviate certain symptoms and slow down the progression of the disease.
D. **Treatments:** No drugs have been developed in order to stop the progression of this disease, however there are medications that can help control the symptoms and prevent complications including:

- **Calcium Channel Blockers:** They affect the movement of calcium in the blood vessels, therefore dilating the blood vessels. This may help to prevent heart, lung, and kidney problems and help to treat Raynaud’s disease, which is very common in patients with scleroderma. Drugs with calcium channel blockers include:
  > Nifedipine  > Amlodipine  > Verapamil  > Felodipine  > Nicardipine
- **Immunosuppressive Medication:** Drugs that suppress the immune system may help to reduce the symptoms associated with Scleroderma. These types of drugs include, but are not limited to:
  > Azathioprine (Imuran)  > Mycophenolate Mofetil (Cellcept)  > Cyclosporine
- **Antacid drugs:** They ease gastro-esophageal disease by reducing stomach acid. Medications such as omeprazole (Prilosec), the most common, can relieve symptoms of acid reflux.
- **Anti-inflammatory Medication:** Muscle pain and weakness associated with scleroderma can be treated with over the counter pain relievers.
- **Physical Therapy:** This helps relieve muscle pain and maintain joint and skin flexibility.
- **Antibiotic Ointment:** Helps prevent infection of fingertip ulcers caused by the Raynaud’s phenomena.

3. **POTENTIAL BENEFITS OF STEM CELL TREATMENT**

Mesenchymal Stem Cells may help to improve skin elasticity and recover some functions severely impaired by this disease. Mesenchymal stem cells exhibit anti-proliferative and anti-inflammatory properties, therefore resetting the immune system and improving the condition and slowing the progression of such disease. T-cells may become induced causing restoration of the regulatory function, leading to disease remission. The therapeutic benefit is due to mesenchymal stem cells locating the site of inflammation and releasing cytokines and growth factors that result in local anti-inflammatory effects.


4. **TREATMENT & DELIVERY METHOD REQUIRED**

A. **Typical Recommended Treatment:** Adipose Derived Stem Cells

B. **Typical Delivery Method Required:** Autologous Ad-SVF containing adult stem cells are infused in 5-10 ml normal saline intravenously with a slow bolus push, as well as with a direct injection of stem cells, resuspended in Platelet Rich Plasma, into the affected areas. A stem cell facial fat transfer may also be recommended.

C. **Recommended dosing:** Recommended repeat dosing MSC’s through IV bolus push every 3 months initially. Also repeat dosing MSC’s combined with PRP every 3 months depending on localized joint pain or stiffness.

5. **POTENTIAL RISKS OF STEM CELL INJECTION(S)**

There are possibilities for unwanted effects related to the local anesthesia, harvesting procedure, and injection of stem cells. Even with the most established protocol, adequate technique, and careful administration; a medical team may encounter uncontrollable events. Although there is no guarantee of any results, excellent results can be attained. The medical professional provides services in the most responsible, professional and diligent manner, always considering that surgeries imply risks. The risks of complications of adipose tissue harvesting and stem cell infusion are very low. Possible risks include but are not limited to:

- Pain at site of injections
- Bleeding at injection site
- Malaise
- Low-grade fever
- Hot flashes
- Itching at injection site
- Vascular spasm or obstruction
- Bruising
- Nerve or muscle injury
- Allergic reaction
- Dizziness
- Nausea
6. FREQUENTLY ASKED QUESTIONS

1. What are adults stem cells and how do they work?
They are undifferentiated cells found in tissues in the body, responsible for maintaining and repairing surrounding cells. These tissues include but are not limited to adipose tissue (fat tissue) and bone marrow. They have the ability to differentiate into various cell types, which is what makes them a potential treatment to alleviate and improve degenerative conditions. It has been proven that there are 500 times more stem cells stored in adipose tissue verse bone marrow, therefore, adipose derived stem cells are far superior.

2. What is Adipose-derived stem cells and what makes it unique?
Adipose-derived stem cells are stem cells found in the adipose tissue of the patient. The adipose tissue is an abundant source of mesenchymal stem cells which have shown the most healing potential. Adipose Stem cells are autologous (patients stem cells), therefore having low risk of immune rejection once the therapy has been completed.

3. How can stem cell therapy help treat Scleroderma?
Scleroderma is an autoimmune disease which affects the elasticity of skin and connective tissues. Autoimmune disease refers to an immune response of the body against tissues and substances that are normally in the body. The cells in a person with scleroderma lose the ability to stop the production of collagen, therefore creating too much collagen and hardening the tissue. Mesenchymal stem cells have shown the ability to suppress proliferation of lymphocytes and restore regulatory functions of T-cells, resulting in anti-inflammatory effects and the reduction of collagen synthesis.

4. How will the stem cells be delivered to the patient?
Intravenous delivery and subcutaneous injection into affected areas, especially hands, face, and feet. This procedure may also include fat transfer into affected areas.

5. How long do you think it would be before I see some improvement?
The response to the treatment varies from patient to patient. Some patients see a response within the first month while other patients take 3-6 months. Many studies have shown patients can improve 6-12 months after a stem cell injection.

6. How long does the procedure take?
The procedure will take approximately 2-3 hours. If you are traveling out of town, you will need to stay in the local area the night of the procedure.

6. SUPPORTING ARTICLES

Adipose tissue-derived stem cells ameliorates dermal fibrosis in a mouse model of scleroderma.
Chen W1, Xia ZK2, Zhang MH2, Ding GC2, Zhang XY2, Wang ZX2, Yang RY3.

Author information
1 Department of Dermatology, Chinese People’s Liberation Army General Hospital, Beijing, China; Department of Dermatology, Zhu Ri He Base Hospital of Beijing Military Command, Inner Mongolia, China; Department of Dermatology, Department of Ultrasound, General Hospital of Beijing Military Command, Beijing, China.
2 Department of Dermatology, Department of Ultrasound, General Hospital of Beijing Military Command, Beijing, China.
3 Department of Dermatology, Department of Ultrasound, General Hospital of Beijing Military Command, Beijing, China.
Electronic address: rongyay=yeah.net.

Abstract

OBJECTIVE: To investigate the therapeutic potential of adipose-derived stem cells (ADSCs) for limited cutaneous scleroderma (LS) in mouse models.

METHODS: ADSCs were isolated from pathogen-free female C57BL/6 mice and LS was induced in wild type (WT) C57BL/6 mice via daily injection of bleomycin (0.1 mL x 300 μg/mL) for 4 weeks; then the ADSCs were subcutaneously injected into the dorsal area in the model treatment group, and 100 µL of phosphate-buffered saline (PBS) solution was injected into the same site in the model control group. Green fluorescent protein (GFP) was used to track the cells using an in vivo imaging system on days 7, 14, 21, and 28 after transplantation.
All mice were sacrificed and histologic analyses were performed after 4 weeks, and the skin thickness, collagen deposition and the total content of hydroxyproline were evaluated. Additionally, immunohistochemistry were performed to compare the tissue expression and distribution of TGF-β1 and VEGF between the ADSCs treatment group and the treatment control group.

**RESULTS:** WT C57BL/6 LS mouse model were successfully established and GFP in vivo fluorescence imaging showed that the translated ADSCs survived at the local for at least 4 weeks. Compared with the control group, the ADSCs treatment group significantly attenuated bleomycin-induced dermal fibrosis, reduced the skin thickness and the total content of hydroxyproline (P < 0.05).
The ADSCs treatment group displayed significantly lower levels of TGF-β1 and higher levels of VEGF than the control group (P < 0.05).

**CONCLUSIONS:** ADSCs may provide a feasible and practical treatment for autoimmune diseases such as LS and ameliorate dermal fibrosis.

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**KEYWORDS:** Adipose-derived stem cells; Limited cutaneous scleroderma; Mouse model; TGF-β1; VEGF

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Adult Stem Cell Treatment Of Scleroderma
Tyndall A., Frust DE
Source: Department of Rheumatology, University of Basel, Switzerland. alan.tyndall@fps-basel.ch

**Abstract**
Around 170 transplanted systemic sclerosis patients are registered in Europe. Most received autologous, peripheral blood derived hematopoietic stem cell transplantation. Treatment-related mortality has fallen to 2.5% in the controlled trials compared with 12.5% in the first report in 2002. Over one-third of patients have experienced sustained remission. Two prospective randomized phase III studies are active: the Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial in Europe and the Scleroderma Cyclophosphamide Or Transplant (SCOT) trial in the USA. Both have similar selection criteria, endpoint and control arms, but the SCOT trial uses radiation and less cyclophosphamide. So far, no unexpected toxicity has occurred. Reports produced in the past 12 months show reduction of skin collagen and reversal of microvascular remodelling, years after transplant. Bone marrow-derived mesenchymal stem cells from systemic sclerosis patients show in-vitro immunomodulatory properties equal to healthy controls.

PMID: 17917542 [PubMed - indexed for MEDLINE]


**Human Adipose-Derived Stromal Cells For Cell-Based Therapies In The Treatment Of Systemic Sclerosis**
Department of Surgery P. Valdani, University Sapienza, Rome, Italy

**Abstract**
The present study was designed to evaluate the clinical outcome of cell-based therapy with cultured adipose derived stromal cells (ASCs) for the treatment of cutaneous manifestations in patients affected by systemic sclerosis (SSc). ASCs have an extraordinary developmental plasticity, including the ability to undergo multilineage differentiation and self-renewal. Moreover, ASCs can be easily harvested from small volumes of liposuction aspirate, showing great in vitro viability and proliferation rate. Here we isolated, characterized, and expanded ASCs, assessing both their mesenchymal origin and their capability to differentiate towards the adipogenic, osteogenic, and chondrogenic lineage. We developed an effective method for ASCs transplantation into sclerodermic patients by means of a hyaluronic acid (HA) solution, which allowed us to achieve precise structural modifications. ASCs were isolated from subcutaneous adipose tissue of six sclerodermic patients and cultured in a chemical-defined medium before autologous transplantation to restore skin sequelae. The results indicated that transplantation of a combination of ASCs in HA solution determined a significant improvement in tightening of the skin without complications such as anechoic areas, fat necrosis, or infections, thus suggesting that ASCs are a potentially valuable source of cells for skin therapy in rare diseases such as SSc and generally in skin disorders.
Repair, Replacement, Regeneration & Reprogramming
The Official Journal of The Cure Alliance

Adipose-Derived Stem Cells: An Innovative Therapeutic Approach In Systemic Sclerosis And Parry-Romberg Syndrome
Vescarelli E., D'Amici S., Onesti M. G., Nodale C., Ceccarelli S., Scuderi N., Angeloni A., Marchese C.

Abstract
Cell-based therapies represent a promising therapeutic approach to enhance the regeneration of damaged tissue and the combination with specific soluble mediators and biomaterial scaffolds has allowed the introduction of new treatment strategies in regenerative medicine. Adipose-derived stem cells (ASCs) show the ability to differentiate into several cell lineages such as chondrocytes, osteoblasts, adipocytes, neuronal cells, and muscle cells. These cells are abundant in normal human fat and they can be easily harvested from small amount of liposuction. For these reasons, they are greatly employed in the treatment of cutaneous and musculoskeletal defects are greatly.

J. M. van Laar1 and A. Tyndall
Adult Stem Cells In The Treatment Of Autoimmune Diseases

Abstract
During the past 10 yrs, over 700 patients suffering from severe autoimmune disease (AD) have received an autologous haematopoietic stem cell transplant as treatment of their disorder with durable remission being obtained in around one-third. The most commonly transplanted ADs have been systemic sclerosis (scleroderma), multiple sclerosis, rheumatoid arthritis, juvenile idiopathic arthritis and systemic lupus erythematosus. A fewer number of patients have received an allogeneic transplant. The initially reported overall treatment-related mortality of 7% has since fallen, with no further cases being reported in systemic sclerosis or multiple sclerosis in the past 3 yrs. This is thought to be due to more careful patient selection. The phase I/II data has led to currently running prospective randomised trials in systemic sclerosis, multiple sclerosis and systemic lupus erythematosus in Europe and North America. Immune reconstitution data suggests a ‘resetting’ of autoimmunity in those patients achieving stable remission, rather than simply prolonged immunosuppression. Recent results from in vitro experiments, animal models and early human experience in severe acute graft vs host disease suggest that multipotent mesenchymal stromal cells obtained from the bone marrow and expanded ex vivo, may exert a clinically useful immunomodulatory effect. Such cells are immune privileged and apparently of low toxicity. Further characterization of these cells and consideration of their possible clinical application in AD is underway.


Autologous adipose-derived stromal vascular fraction in patients with systemic sclerosis: 12-month follow-up
Perrine Guillaume-Jugnot1,*, Aure´lie Daumas1, Jean-Pierre Magalon2, Elisabeth Jouve3, Pierre-Se´bastien Nguyen4, Romain Truillet3, Stéphanie Mallet5, Dominique Casanova4, Laurent Giraudo2, Julie Veran2, Francine Dignat-George6,7, Florence Sabatier2,6,7, Guy Magalon3,4 and Brigitte Graneli1,7

Abstract
Objective. Impaired hand function greatly contributes to disability and reduced quality of life in SSc patients. Autologous adipose-derived stromal vascular fraction (ADSVF) is recognized as an easily accessible source of regenerative cells. We reported positive 6-month safety and efficacy results from an open-label clinical trial assessing s.c. injection of autologous ADSVF into the fingers in SSc patients. The objective of this report is to describe the effects at 12 months.

Methods. Twelve females, mean age 54.5 years (S.D. 10.3), were assessed 1 year after ADSVF injection. Patients were eligible if they had a Cochin Hand Function Scale score >20/90. ADSVF was obtained from lipoaspirate using an automated processing system and subsequently injected into the s.c. tissue of each finger in contact with neurovascular pedicles in a one-time procedure. Endpoints were changes in hand disability and skin fibrosis, vascular manifestations, pain and quality of life at the 12 month follow-up. During the visit, patients estimated the benefit of the procedure with a specific self-completed questionnaire.
Results. A significant decrease from baseline of 51.3% (P<0.001) for Cochin Hand Function Scale score, 63.2% (P<0.001) for RP severity and 46.8% (P = 0.001) for quality of life (Scleroderma Health Assessment Questionnaire) was observed. A significant improvement of finger oedema, skin sclerosis, motion and strength of the hands and of the vascular suppression score was also noted. The reduction in hand pain approached statistical significance (P = 0.052). The questionnaire revealed a benefit in daily activities, housework and social activities.

Conclusion. ADSVF injection is a promising therapy and appears to have benefits that extend for at least 1 year.