New strategy and possibility for using ADRCs for Treating Scleroderma

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FROM FAT TO STEM CELLS
Treatment for FACE and HANDS in SCLERODERMA

1996
SYDNEY COLEMAN
LIPOSTRUCTURE ®

2009
MICROFAT GRAFTING
FACE SCLERODERMA PATIENTS

2012
SVF INJECTION
HANDS SCLERODERMA DISABILITY

ATMP
Advanced Therapy Medicinal Products

Ø 2 mm
P. NGUYEN
FACE ASSESSMENT IN PATIENTS WITH SCLERODERMA
Facial autologous fat grafting

• Indications
  ▪ Reconstructive surgery
    • Traumatic sequelae
    • Burns
    • Radiation therapy,
    • Iatrogenic lipodystrophy
    • Facial hemiatrophy ...
  ▪ Aesthetic surgery
    • Filling and reduction of wrinkles
    • Restoring facial contours and face volumes

• In systemic sclerosis (SSc)
  – One case in systemic sclerosis
    « Deep phenol peeling and fat injection: treatment option for perioral wrinkles in a scleroderma patient. »

  – « En coup de sabre » scleroderma
    « Frontallinear scleroderma: long-term result in volumetric restoration of the fronto-orbital area by structural fat grafting. »

  – Peri oral fat injection. Average mouth opening
    « Autologous fat grafting in the treatment of fibrotic perioral changes in patients with systemic sclerosis. »

  – Facial treatment on Systemic Sclerosis Patients
    « Efficacy of Autologous Microfat Graft on Facial Handicap in Systemic Sclerosis Patients. »
    Nolwenn Sautereau, Aurelie Daumas, Romain Truillet, Elisabeth Jouve, Jeremy Magalon, Julie Veran, Dominique Casanova, Yves Frances, Guy Magalon, Brigitte Granel. PRS Global Open • 2016
1. Fat harvesting : 10-30 min

2. Fat purification: 15 min

3. Fat delivery : 5-10 min
MICRO INJECTION

HARVESTING CANNULA – CLOSED SYSTEM

Ø=2mm – 14 Gauge – 130mm

S=0.58 mm²

PURIFICATION

INJECTION CANNULA

Ø=0.8mm – 21 Gauge – 4mm
Treatment of Scleroderma injuries of the face using a mixture of Fat and PRP
<table>
<thead>
<tr>
<th>MHISS score (/48)</th>
<th>Inclusion (M0)</th>
<th>6 months (M6)</th>
<th>Variation M0-M6</th>
<th>12 months (M12)</th>
<th>Variation M0-M12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (±SD)</td>
<td>32.6 (± 6.3)</td>
<td>21.8 (± 8.9)</td>
<td>-10.7 (± 5.1) p&lt;0.0001</td>
<td>22.8 (± 8.9)</td>
<td>-9.8 (± 6) p=0.0002</td>
</tr>
</tbody>
</table>

**MHISS score**

![Graph showing changes in MHISS score, mouth opening, oral sicca syndrome, and aesthetics over time.](image)
Aesthetics: perioral radial folds improvement

59 years old, diffuse cutaneous form

67 years old, limited cutaneous form
Aesthetics: mouth opening

55 years old
Limited cutaneous form

65 years old
Diffuse cutaneous form
Patient’s satisfaction

Same satisfaction at 6 and 12 months

- 4 Very satisfied
- 5 Satisfied
- 2 Moderately satisfied
- 1 Unsatisfied

Same satisfaction at 6 and 12 months
HANDS ASSESSMENT IN PATIENTS WITH SCLERODERMA
Cells are immediately available after a brief modification

**STROMAL VASCULAR FRACTION**

**SVF**

**CENTRIFUGATION +/- DIGESTION COLLAGENASE**

**FAT LIQUIFICATION**

**STROMAL VASCULAR FRACTION**

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**Multilineage Cells from Human Adipose Tissue: Implications for Cell-Based Therapies**

*TISSUE ENGINEERING* Volume 7, Number 2, 2001

**Adipose-Derived Stem Cells in Tissue Regeneration: A Review**

Patricia Zuk

Regenerative Bioengineering and Repair Laboratory and Division of Plastic and Reconstructive Surgery, Department of Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

ISRN Stem Cells, Volume 2013 (2013), Article ID 713959, 35 pages

http://dx.doi.org/10.1155/2013/713959
Safety, tolerability and potential efficacy of injection of autologous adipose-derived Stromal Vascular Fraction in the fingers of patients with systemic sclerosis: An open-label phase I trial.


A service of the U.S. National Institutes of Health
ClinicalTrials.gov
« Assessment of the Subcutaneous Reinjection of Human Autologous Adipose-derived Stromal Vascular Fraction (Celution® System) in the Hands of Patients Suffering From Systemic Sclerosis »

Systemic Sclerosis
The Cell Therapy
Phase I – II
- Screening for safety
- Establishing the testing protocol
### APHM multidisciplinary team

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daumas A, Serratrice J, Swiader L, Weiller PJ, Rossi P, Frances Y, Granel B.</td>
<td>Internal Medicine</td>
</tr>
<tr>
<td>Samson D.</td>
<td>Anaesthesia and Reanimation Department</td>
</tr>
<tr>
<td>Baïada A.</td>
<td>Physiotherapy</td>
</tr>
<tr>
<td>Petit P, Colavolpe N.</td>
<td>Medical Imaging</td>
</tr>
<tr>
<td>Mallet S.</td>
<td>Dermatology</td>
</tr>
<tr>
<td>Sabatier F, Veran J, Giraudo L, Arnaud L, Aboudou H, Roussey A, Dignat-George F.</td>
<td>Laboratory of Cell Therapy and Clinical Investigation, Laboratory of Hematology and Vascular Biology</td>
</tr>
<tr>
<td>Jouve E, Charles E, Reynier JCh.</td>
<td>CPCET - Therapeutic Evaluation Center</td>
</tr>
</tbody>
</table>
Hands: the visible and obvious signs of the disease

HOW TO ASSESS THESE HANDS?
INTEREST OF AUTOLOGOUS ADIPOSE TISSUE ON THE RAYNAUD’S PHENOMENON

« Fat Grafting to the Hand in Patients with Raynaud Phenomenon: A Novel Therapeutic Modality »

Jonathan Bank, M.D., Sam M. Fuller, M.D., Ginard I. Henry, M.D.
Lawrence S. Zachary, M.D.

Plastic and Reconstructive Surgery • May 2014, vol 133, number 5

Conclusions: Preliminary results of fat grafting to the hands of patients with Raynaud phenomenon revealed improved symptomatology with evidence suggestive of measurably increased perfusion in some cases. Fat grafting may benefit the management of this patient population. (Plast. Reconstr. Surg. 133: 1109, 2014.)
INTEREST OF AUTOLOGOUS ADIPOSE TISSUE IN TREATMENT OF ISCHEMIC DIGITAL ULCERS (DU) IN SYSTEMIC SCLEROSIS

« Regional implantation of autologous adipose tissue-derived cells induces a prompt healing of long-lasting indolent digital ulcers in patients with Systemic Sclerosis »

Nicoletta Del Papa; Gabriele Di Luca; Domenico Sambataro; Eleonora Zaccara; Wanda Maglione; Armando Gabrielli; Paolo Fraticelli; Gianluca Moroncini; Lorenzo Beretta; Alessandro Santaniello; Gianluca Sambataro; Roberto Ferraresi; Claudio Vitali

Cell Transplantation, 2014

« Fifteen patients with SSc having a long-lasting DU in only one fingertip, unresponsive to intensive systemic and local treatment, were enrolled in the study. The grafting procedure consisted in the injection, at the basis of the corresponding finger, of 0.5-1 ml of autologous ATDCs fraction. »
INTEREST OF AUTOLOGOUS ADIPOSE TISSUE DERIVED STROMAL VASCULAR FRACTION (ADSVF) IN THE TREATMENT OF HANDS IN SYSTEMIC SCLEROSIS PATIENTS

« Safety, tolerability and potential efficacy of injection of autologous adipose-derived stromal vascular fraction in the fingers of patients with systemic sclerosis: an open-label phase I trial. »


Ann Rheum Dis. 2014 Aug 11

This study outlines the safety of the autologous SVF cells injection in the hands of patients with SSc. Preliminary assessments at 6 months suggest potential efficacy needing confirmation in a randomised placebo-controlled trial on a larger population.
<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>STUDY CHARACTERISTICS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indication</td>
<td>Raynaud phenomenon</td>
<td>SSc</td>
<td>SSc</td>
</tr>
<tr>
<td>Number of patients</td>
<td>13</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Number of hands/DUs treated</td>
<td>21 hands</td>
<td>15 Digital ulcers</td>
<td>24 hands</td>
</tr>
<tr>
<td><strong>INJECTED PRODUCT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvested volume of fat (ml)</td>
<td>Not precised</td>
<td>Not precised</td>
<td>174 ± 46</td>
</tr>
<tr>
<td>Preparation</td>
<td>Decantation - 5 hands Lipivage® -16 hands</td>
<td>Centrifugation Coleman Procedure</td>
<td>Enzymatic digestion (Stromal Vascular Fraction) Cytory’s Celution®</td>
</tr>
<tr>
<td>Adipocytes</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Number of SVF cells</td>
<td>Not precised</td>
<td>Not precised</td>
<td>YES 3.76 millions per finger</td>
</tr>
<tr>
<td>Volume (cc)</td>
<td>30 cc per hand</td>
<td>0.5 -1 cc per finger affected</td>
<td>1 cc per finger</td>
</tr>
</tbody>
</table>
12 Patients – 24 HANDS

CLINICAL EXAMINATION
• **Cochin** hand functional disability scale - **CHFS**
• Scleroderma Health Assessment Questionnaire - **SSc – HAQ**
• Hand mobility in scleroderma - **Hamis Test**
• **Rodnan** skin score and **Rodnan** score focused on hand
• Evaluation of **Raynaud**’s syndrom
• Visual Analogic pain Scale for the hand - **VAS**

PARACLINICAL EXAMINATION
• X-ray
• Doppler : Ulnar and radial arteries
• Laser Doppler tissue imaging
• Capillaroscopy
THE COCHIN SCALE

Answers to the questions: 18

0 = Yes, without difficulty

1 = Yes, with a little difficulty

2 = Yes, with some difficulty

3 = Yes, with much difficulty

4 = Nearly impossible to do

5 = Impossible to do

Maximum score = 90

Osteoarthritis and Cartilage (2001) 9, 570–577
© 2001 OsteoArthritis Research Society International
Reliability, validity, and sensitivity to change of the Cochin hand functional disability scale in hand osteoarthritis
<table>
<thead>
<tr>
<th>In the kitchen</th>
<th>Dressing</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Can you hold a plate full of food?</td>
<td></td>
<td>17. Can you pick up coins from a table top?</td>
</tr>
<tr>
<td>4. Can you pour liquid from a bottle into a glass?</td>
<td></td>
<td>18. Can you turn a key in a lock?</td>
</tr>
<tr>
<td>5. Can you unscrew the lid from a jar opened before?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Can you cut meat with a knife?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Can you prick things well with a fork?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Can you peel fruit?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Can you button your shirt?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Can you open and close a zipper?</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hygiene</strong></td>
<td><strong>At the office</strong></td>
<td></td>
</tr>
<tr>
<td>12. Can you hold a toothbrush efficiently</td>
<td>14. Can you write a letter with an ordinary pen?</td>
<td></td>
</tr>
<tr>
<td>15. Can you turn a round door knob?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. Can you cut a piece of paper with scissors?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. Can you pick up coins from a table top?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. Can you turn a key in a lock?</td>
<td></td>
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</tbody>
</table>
CHARACTERISTICS OF PATIENTS AND DISEASE

• 12 females with systemic scleroderma, mean age 54.5 years (38-64)
• 7 cutaneous limited (58.33%) and 5 cutaneous diffuse (41.67%)
  – Disease duration : 9.9 years (2-24)
• Raynaud’s syndrome : 100%
  – Disease duration : 14.3 years (5-34)
• Digestive symptoms : 75 %
• Respiratory symptoms : 83.3%
• Cardiac symptoms : 8.3 %
• Sicca syndrome : 33.3%
QUALITY CONTROLS

- STERILITY TESTING
- CELL NUMERATION AND VIABILITY
- FUNCTIONAL ASSAY
  - CFU-F (Colony Forming Unit–Fibroblats)
- FLOW CYTOMETRY ANALYSIS OF CELL POPULATIONS
## Cell Dose Information

<p>| | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td><strong>Volume of Fat Harvested</strong></td>
<td>174 ± 46 mL</td>
</tr>
<tr>
<td><strong>Viable Cell Yield</strong></td>
<td>50.4 ± 24.7 x 10^6</td>
</tr>
<tr>
<td><strong>Cell Dose Delivered per Finger</strong></td>
<td>3.7 ± 1.8 x 10^6</td>
</tr>
<tr>
<td>% CD45^+/CD34^-/CD146^- cells (leukocytes)</td>
<td>49 ± 18</td>
</tr>
<tr>
<td>% CD45^-/CD34^{bright}/CD146^{dim} cells (EPC-like)</td>
<td>3.4 ± 2.2</td>
</tr>
<tr>
<td>% CD45^-/CD34^{dim}/CD146^{bright} cells (endothelial)</td>
<td>6.4 ± 6.8</td>
</tr>
<tr>
<td>% CD45^-/CD34^{bright}/CD146^-/CD90^+ cells (MSC-like)</td>
<td>36 ± 14.5</td>
</tr>
<tr>
<td>% CD45^-/CD34^{bright}/CD146^-/CD90^+ cells (HSC-like)</td>
<td>5.1 ± 2.3</td>
</tr>
<tr>
<td>% Fibroblast-like cell Colony-Forming Unit (CFU-F)</td>
<td>3.7 ± 1.9</td>
</tr>
</tbody>
</table>

All samples sterile by gram stain and by Bactec™ testing for both aerobic and anaerobic organisms
**AP-HM** (Assistance Publique des Hôpitaux de Marseille) **strategy** based on recommendations of **ANSM** (Agence Nationale de la Santé du Médicament)/**EMA** (European Medicines Agency)

**2012 – SCLERADEC 1 - clinical trial**

<table>
<thead>
<tr>
<th>Markers combination</th>
<th>Identified Populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD90-FITC</td>
<td>CD45-PC5 DRAQ5 DAPI</td>
</tr>
<tr>
<td>CD146-PE</td>
<td></td>
</tr>
<tr>
<td>CD34-ECD</td>
<td></td>
</tr>
</tbody>
</table>

1- Leukocytes
2- Endothelial cells
3- Stromal cells

**2016 – SCLERADEC 2 - clinical trial**

<table>
<thead>
<tr>
<th>Tube 1</th>
<th>Tube 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD90-FITC</td>
<td>CD14-FITC</td>
</tr>
<tr>
<td>CD146-PE</td>
<td>CD34-ECD</td>
</tr>
<tr>
<td>CD34-ECD</td>
<td>CD45-PC5</td>
</tr>
<tr>
<td>CD45-PC5</td>
<td>CD56-PC7</td>
</tr>
<tr>
<td>DAPI</td>
<td>CD3-AA750 DAPI</td>
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</tbody>
</table>

1- Pericytes
2- Transitional cells
3- Endothelial cells
4&5- Stromal cells
6- CD34+ macrophages
7- Granulocytes
8- Monocytes
9- NK cells
10- T lymphocytes
11- Others lymphocytes
## POST OPERATIVE CONTROLS

<table>
<thead>
<tr>
<th></th>
<th>J1</th>
<th>J7</th>
<th>J21</th>
</tr>
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<tbody>
<tr>
<td><strong>HANDS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitive disorders</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hematomas</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infection</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Need for amputation</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>HARVESTING AREA</strong></td>
<td></td>
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</tr>
<tr>
<td>Hematomas</td>
<td>1</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Infection</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>

**NO SERIOUS ADVERSE EVENT**
Improvement in Hand Strength

**Objective Improvement in Grip and Pinch Strength: Dynamometer**

**Grip Strength**

<table>
<thead>
<tr>
<th>Normal Range 15kg-39kg¹</th>
<th>Baseline</th>
<th>2 Months</th>
<th>6 Months</th>
<th>12 Months</th>
<th>24 Months</th>
</tr>
</thead>
<tbody>
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<td></td>
<td><img src="grip_strength_graph.png" alt="Graph" /></td>
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</table>

**Pinch Strength**

<table>
<thead>
<tr>
<th>Normal Range 3.5kg-7.7kg¹</th>
<th>Baseline</th>
<th>2 Months</th>
<th>6 Months</th>
<th>12 Months</th>
<th>24 Months</th>
</tr>
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<td><img src="pinch_strength_graph.png" alt="Graph" /></td>
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</table>

Data presented as mean ± standard error

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Digital Ulcers

~60% decrease in total number of digital ulcers at 24 months

24 months follow-up for SSc patients after SVF re-injection

October 2016 - Current Research in Translational Medicine – Brief communication
Long-term follow-up after autologous adipose-derived stromal vascular fraction injection into fingers in systemic sclerosis patients
Autologous adipose-derived stromal vascular fraction in scleroderma
A Daumas, J Magalon, E Jouve, R Truillet, D Casanova, L Giraudo, J Veran, A Benyamine, F Dignat-George, G Magalon, F Sabatier, B Granel
Patient Comments

“It felt like ten fingers had been grafted on to me”

“There is no longer any pain at all”

“I’m living again”

“My sense of touch has improved”

“My fingers are pinker”
Conclusion

• **Microfat grafting** has been developed to treat **Scleroderma Faces**. This is an efficient microinvasive painless procedure with long term results.

• Injection of autologous of **Stromal Vascular Fraction** into **the Hands** can be performed safely. SVF treatment was associated with an 50% improvement of Cochin hand function scale, Raynaud’s condition score and global disability at two years. Longer term, larger and controlled studies will be important to confirm whether this new form of cell therapy can improve the long term prognosis.

• Controlled and comparative studies are needed to confirm the promising results obtained par both autologous **Fat grafting and SVF injection procedure** in open studies

• **Next steps**: **2 clinical trials, SVF**
  - **France**: **SCLERADEC II**, randomized, 10 to 70 millions cells, 5 Centers, 40 Cases – **In progress 25 cases**
  - **USA**: **STAR**, randomized, 40 millions cells, 15 States, 88 Cases – **Completed**
  - **European project**: **FAST**, randomized, cryopreserved products, 4 countries - **Waiting**