Tissue Regeneration in Reconstructive and Aesthetic Surgery: Platelet Grow Factors, Lipotransfer and Implantation of Fat Derived Stem Cells

Regenerative surgery deals with damaged tissue via endogenous cells activation or through autologous cells implantation. It is a new therapeutic approach devoted to a biological restoration of tissues without their substitution only using fresh autologous cells and/or cell derivatives. It might be also obtained with an *in vitro* multiplication of stem cells or autologous cell precursors taken from a donor tissue/organ. In the last case they might be used in *tissue engineering* which is employing in vitro cultivations and reconstructions of tissue and organs using polimeric and biocompatible tridimensional matrixes.

Regenerative Techniques: our experience 2004-2010

1. **PLATELET GEL**: Plasma rich platelet derivatives
2. **LIPOFILLING** and LIPOSTRUCTURE: for Fat Graft
3. **STEM CELLS from ADIPOCYTES**: Fat grafts enriched with Stem Cells obtained from adult adipocytes

1. **Regenerative Techniques using Platelet Derivatives**: our experience 2004-2010

• **PLATELET GEL**: using only Platelet Growth Factors (PGFs)
• **PLATELET GEL+LIPOSTRUCTURE**: Platelet Growth Factors (PGFs) associated to an adipose tissue implant
• **PLATELET GEL+HA**: Platelet Growth Factors (PGFs) associated to sheets of Hyaluronic acid (HYAFF)
It is a technique which allow us to use growth factors contained inside platelet granules in the form of Platelet Rich Plasma (PRP) to accelerate healing processes for bones and soft tissues. Growth Factors may be divided in:

- **Initial**: bFGF, PDGF, IGF
- **Late**: EGF, VEGF, TGF-b, IGF

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### Platelet Gel: from theory to practice

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>From 10 ml of blood we may obtain 2 ml of platelet rich plasma (LIQUID FORM)</td>
</tr>
<tr>
<td>2</td>
<td>Adding 0.5 ml of a CaCl solution for each ml of the liquid form</td>
</tr>
<tr>
<td>3</td>
<td>CaCl solution ACTIVATE the liquid form which tends to CONSOLIDATE and BECOME a GEL in 5 minutes</td>
</tr>
<tr>
<td>4</td>
<td><strong>After second centrifugation at 1100 rate x minutes for other 5 minutes</strong></td>
</tr>
<tr>
<td>5</td>
<td>We obtain a more SOLID FORM of Platelet gelification</td>
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### ADVANTAGES

- Stop of bleeding
- Reduction of biomaterial dispersion by exudation
- Quicker maturation of grafts
- Quicker mineralization of bones substitutes
- Quicker cicatrization
- Lower risk of graft dehiscence and bacterial contamination

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### Use of Platelet Gel in Plastic Surgery

<table>
<thead>
<tr>
<th>Name</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Lións</td>
<td>47</td>
</tr>
<tr>
<td>Cecatri</td>
<td>4</td>
</tr>
<tr>
<td>S. Rosenb</td>
<td>5</td>
</tr>
<tr>
<td>Ball de Uzedo</td>
<td>59</td>
</tr>
</tbody>
</table>
Use of Platelet Gel in Maxillo-Facial Surgery

RESULTS:

86% of the treated patients showed a quicker reparation of soft tissues compared to the control group.

14% of treated patients showed a healing time of soft tissues equal to the one of the control group.

Use of Platelet Gel in Plastic Surgery

RESULTS:

67% of the treated patients showed a quicker reparation of soft tissues compared to the control group.

33% of the treated patients showed an healing time of soft tissues equal to the one of the control group.

PLATELET GEL:

CLINICAL CASES
LEG ULCERS

NOWADAY AVAILABLE ALTERNATIVES

1. AUTOLOGOUS SKIN

2. BANK Skin (omologous skin from cadaveric donors)

3. ENGINEERED SKIN (Autologous or Omologous skin vitro expanded)

4. SYNTETIC BIOMATERIALS

LEG ULCER
PRE-OPERATORY

LEG ULCER
INTRA-OPERATIVE
LEG ULCER

INTRA-OPERATIVE

IMMEDIATE POST-OP.

EXTERNAL MEDICATION WITH HYAFF

POST-OP. 7 DAYS LATER

Cervelli V, Gentile P, Grimaldi M.


PMID: 19156458
2. Regenerative Techniques using Lipofilling and lipostructure: our experience 2004-2010

- **LIPOTRANSFER**: basic fat graft
- **LIPOSTRUCTURE**: Sidney Coleman Technique for fat grafting
LIPOFILLING and LIPOSTRUCTURE: COMPARISON between the two TECHNIQUES

Fat collection is made through normal cannulas for liposuction and a syringe.

LIPOFILLING

LIPOSTRUCTURE

Fat collection is done through sububile cannulas (2mm), less thraumatic for tissues.

LIPOFILLING

Collected fat is left for decantation on three levels.
In the upper layer we found lipids which are the result of the adipocyte disruption.

In the yellow central layer we found the remaining of entire adipocytes.

In the pink lower layer we found blood and sediment and liquids resulting from pre-operative infiltration.

After collection the aspirated is immediately put in a centrifuge for 3 minutes at 3000 rpm x min.
- After centrifugation collected materials is more clearly divided in:
  - oil
  - adipocytes
  - serum
  - destroyed eritrocytes
  - eritrocytes

**LIPOFILLING**

*Fat random distribution in one layer*

**LIPOSTRUCTURE**

Fractioned deposit, in form of beans of necklas, in several tunnels and in different layers

**LIPOSTRUCTURE**

Fat deposit on several layers
Factors influencing the long-term survival of fat transfer:

- Blood flow of receiving site
- Extensive manipulation of donor fat
- High negative pressure during the uptake
- Diameter of fat fragments
Factors influencing the long-term survival of fat transfer

excess of fat volume followed by central necrosis

AUTOLOGOUS FAT TRANSPLANT INDICATIONS

Volumetric tissue deficiency due to congenital or acquired pathology of face and body

AUTOLOGOUS FAT TRANSPLANT INDICATIONS

Congenital defects

- Parry Romberg Syndrome
- Poland Syndrome
- Congenital Facial Atrophy
- Distrettual Ipotrophy

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**Acquired Defects**

- post-trauma result
- burn result
- cutaneous flap result
- mastectomy result
- "other" pathology long term result
- radio-therapy ulcer

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**Romberg Syndrome**

Left face atrophy

The pathogenesis of this syndrome is not clear. Some authors attribute the atrophy of the subcutaneous tissue to an alteration of the autonomic system. Others attribute it to an alteration of the nervous system or to an evident result of the atrophied sign [1, 2]. This disease is characterized by atrophy of the subcutaneous tissue, most frequently on the left side of the face. In advanced forms of the syndrome, the face is very thin, and the distribution of the subcutaneous tissue is very diffuse and atrophic. The most frequent sign is loss of function of the face. The face is thin, of three-dimensional development (atrophy of the subcutaneous tissue). It is considered a hereditary disease of type A from Italy.
We used lipostructure + PRP in the following quantity for each area:

- 15 cc  nose-buccal line
- 15 cc  zygomatic area
- 45 cc  left cheek
- 5 cc   left eyebrow
- 15 cc  left temporal area
- 3 cc   supra-orbital area
- 7 cc   mental area
CHEMICAL BURN SEQUELAE
**OTHER CLINICAL CASES**

- Burn sequela, treated with lipostructure (40cc).
- Post-traumatic scar, treated with lipostructure (50cc).
Other clinical cases:

- Mammary reconstruction sequela, treated with lipostructure (100cc).
- Lipodystrophy sequela, treated with lipostructure (20cc).
- Lipodystrophy sequela, treated with lipostructure (30cc).
- Post-traumatic depression, treated with lipostructure (100cc).
CASI CLINICI

lipodistrophy sequela, treated with lipostructure (30cc) and cervico-facial lifting.

Stem Cells in Human Adipose Tissue

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

Human Adipose Tissue Is a Source of Multipotent Stem Cells

Stem Cells Are Derived from the Stromal Fraction

Stem Cell Content: Marrow vrs Adipose Tissue

<table>
<thead>
<tr>
<th>Stem Cell Frequency (%)</th>
<th>0.0004%</th>
<th>1-2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>500,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100,000</td>
<td></td>
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</tr>
</tbody>
</table>

Bone Marrow Adipose Tissue

Enzymatic Digestion

Aspirated Adipose Tissue

Culture Stromal Fraction

ADSC

Molecular Biology of the Cell
Vol. 13, 4279–4295, December 2002
Perhaps cell culture isn’t necessary to get a therapeutic dose.

Clinical Case Reports
- 50 yo Italian woman with non-healing sternal ulcer secondary to radiotherapy for lymphoma

Outcome

Summary
- Adult stem cells have the potential to be valuable in wound repair
- The frequency and yield of stem cells in digested human adipose tissue may be sufficient for treatment without the need for cell culture
- Early clinical data from investigator-initiated studies show promising results in radiotherapy-related wounds
Where is the future?

In the last ten years it has become apparent that there is a large concentration of stem cells and precursors cells in fat. Since fat is available in all humans and easily accessible the race for stem cells has begun.

Embryonic Stem Cells
- They are developmental cells; not repair cells.
- Their native function is to create an organism not repair it.
- This property is why they form teratomas when implanted.

Adult Stem Cells
- They are a key part of the body repair process.
- Their native function is to be a local source of new functional cells to replace those lost to daily wear and tear, injury, and disease.

Most of all tissues contain a population of stem cells. Their function is to replace matures cells that die or are lost to injury etc. They can also form new stem cells of the same kind.
Where is the future? The Source

Mesenchimal Derived Stem Cells
Etherogenic derived from bone marrow
Laboratory duplicated by tissue culturing
Potential Graft vs Host reaction

Adipose Derived Stem Cells
Autologous derived from the adipose tissue
Produced on line, fresh during surgery
No reaction vs host

Where is the future? Adipose tissue is the ideal source

• Autologous-Use
  No known risk of rejection
  No known risk of disease transmission
  No Ethical Concerns
  Easier regulatory issues

Fat Grafting
Where is the future? How to extract stem cells from the fat

The way to extract stem cells from the fat
Fully automated (Cytori)
Manual (CAL)

Celution System -CYTORI-
ADSCs mixed with Fat B and implanted + ADSCs mixed with Fat A and implanted

Mammary reconstruction using ADSCs

Cannulas

Only washing

Enzymatic digestion

Fat B

Fat A

1° CICLE
Fat Grafting
Where is the future? How do stem cells influence the process of take?

At the adipocytes level, the ADRC cells express factors that modulate inflammation and stimulate angiogenesis like VEGF which increases vascular permeability and leukocyte extravasation.
Activation and proliferation of the endothelium. Vessel sprouting.

Fat Grafting
Where is the future? How do stem cells influence the process of take?

CLINICAL CASES
Preop. 7 weeks postop.

The Present of Fat Grafting
Aesthetic Surgery. The Breast

Pre op
6 months FU

Brava System
Aspiration Breast Holder

Progressive breast increasing after 3 weeks of application
The Present of Fat Grafting
Aesthetic Surgery. The Breast

Pre op
After Brava

Brava System
Aspiration Breast Holder
Progressive breast increasing after 3 weeks of application with the presence of a natural scaffold between the skin and the gland.

The Present of Fat Grafting
Aesthetic Surgery. The Breast

Pre op
6 months FU
The Present of Fat Grafting
Aesthetic Surgery. The Breast

Fat injected 180 cc
1 Session

PRE OP.

3 MONTHS

234 cc (R) 240 cc (L)

Villa Mondragone International School
SECOND INTERNATIONAL CONFERENCE
REGENERATIVE SURGERY

October 27-28-29-30, 2010 - Rome, Italy