

Il futuro della medicina rigenerativa ed i vari ambiti di applicazione

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Perianal Complex Crohn's disease

The Burden of the Disease

il suo nome detto questa
notte mette già paura

- ✓ Over **150.000 pts** in Europe and USA (10 % pediatric) – ≈ **12.000 in Italia**
- ✓ **Costs: ≈ 25.000 € / year** (direct and indirect)
- ✓ Greatly **diminished HR-QOL** (pain, discharge, relation, sexual, work)
- ✓ **Impaired healing of tissues** (inflammation, malnutrition, medical therapies)
- ✓ Outcome depends on **location and activity of systemic disease**
- ✓ **Surgical treatment alone** is effective in relieving symptoms and sepsis control but is associated with **low healing rates**
- ✓ Significant **risk of permanent stoma** (12-42 %) (**anorectal stenosis, cavitating ulcers**)
- ✓ Don't forget the **risk of neoplastic degeneration** (> long standing disease)
- ✓ **Biologic agents significantly modified the therapeutic algorithm**

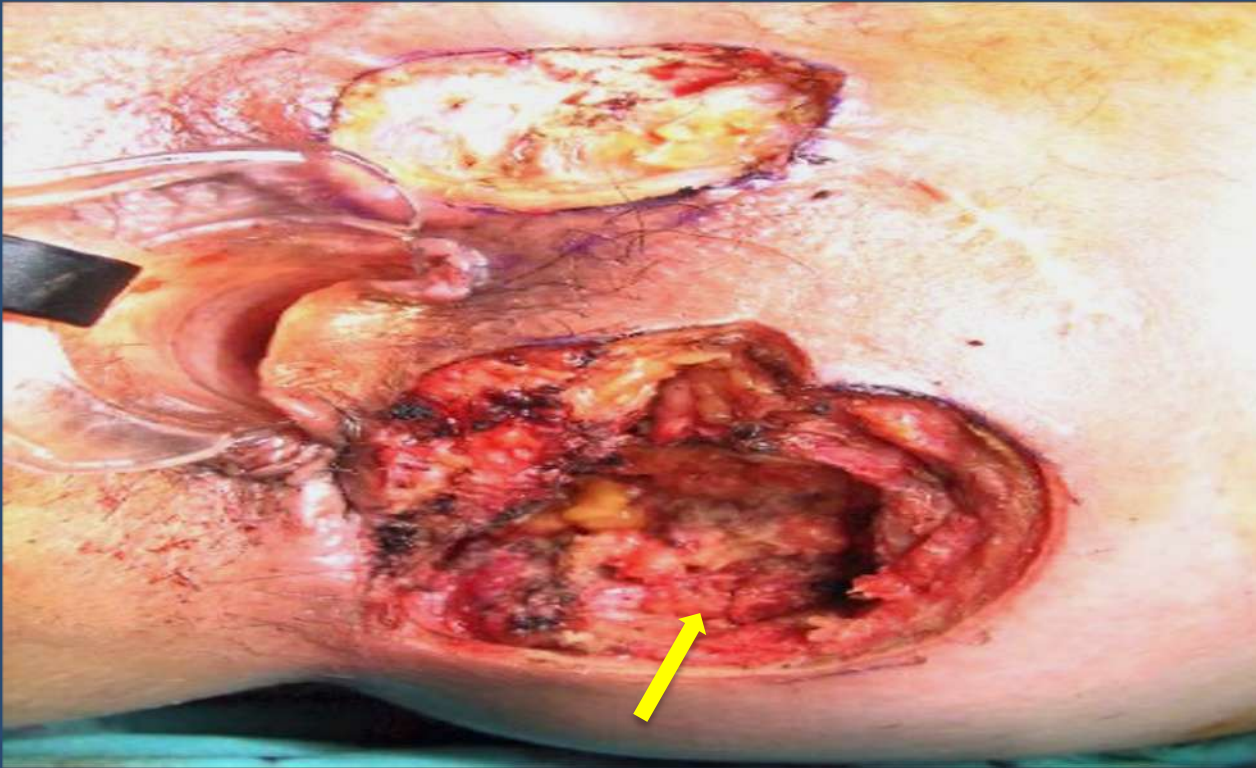
Complex Perianal Fistulas in CD



SUGGESTED

1° step: Sanitization of perianal sepsis

“Cone-like” Fistulectomy and loose seton placement



BRIDGING TREATMENT

Convert an acute uncontrolled situation into a potentially curative situation



Complex Perianal Crohn's Disease

“ENDOANAL MUCOSAL FLAP”

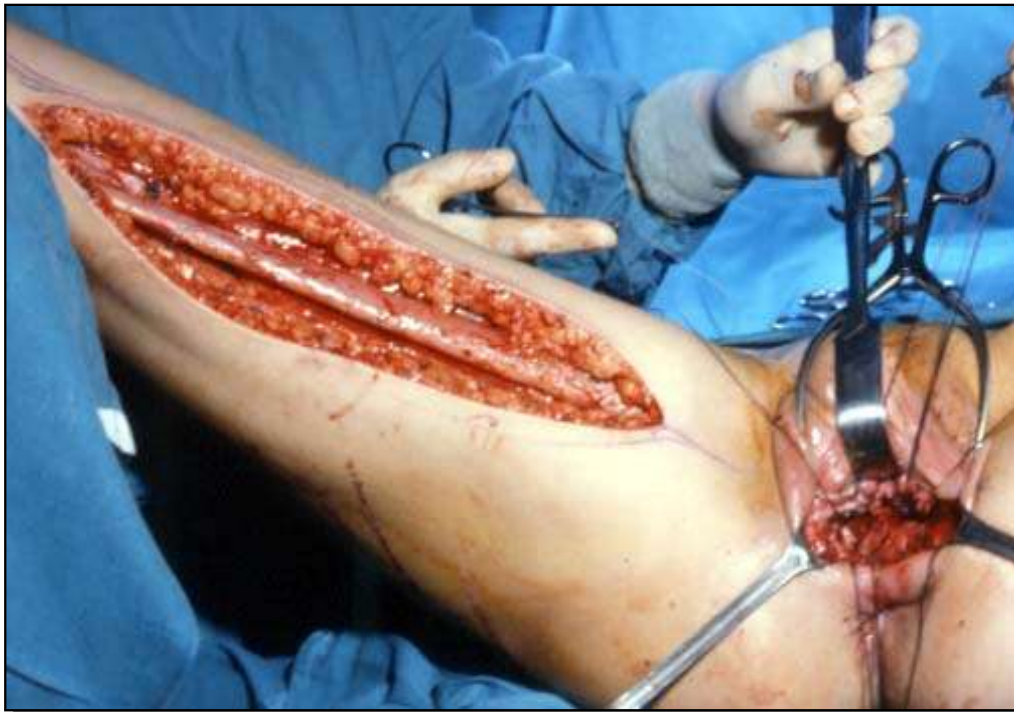


Unità di Chirurgia del TA - Bologna, 1985-2023

Fistole ano-vaginali

48 pz

GRACILOPLASTICA



Approccio originale



Nuovo approccio

3. EUROPEAN Evidence-based consensus on the diagnosis and management of Crohn's disease 2016: Part 2: Surgical management and special situations

* Paolo Gionchetti, *Axel Dignass, Silvio Danese, Fernando José Magro Dias, Gerhard Rogler, Péter Laszlo Lakatos, Michel Adamina, Sandro Ardizzone, Christianne J. Buskens, Shaji Sebastian, Silvio Laureti, Gianluca M. Sampietro, Boris Vucelic, C. Janneke van der Woude, Manuel Barreiro-de Acosta, Christian Maaser, Francisco Portela, Stephan R. Vavricka, Fernando Gomollón, on behalf of ECCO (* PG and AD contributed equally to this work, AD, FG and PG acted as convenors of the consensus)



ECCO statement 9I

In complex perianal fistulising diseases
adequate surgical drainage *if indicated*.

BUT

medical therapy following

ECCO Statement 9J

Imaging before surgical drainage is recommended. EUA for surgical drainage of sepsis is **mandatory for complex fistulas** [EL 4]. In complex fistulas, abscess drainage and loose seton placement should be performed [EL4]

ECCO Statement 9G

Seton placement after surgical treatment of sepsis is recommended for complex fistulae [EL2]. The timing of removal depends on subsequent therapy



Complex Perianal Crohn's Disease

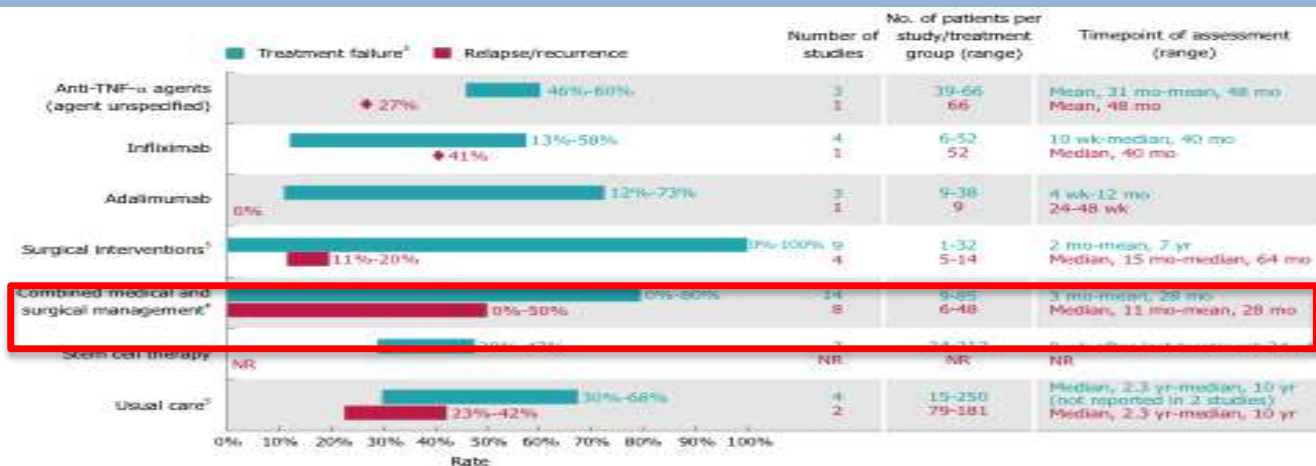
The Burden of the Disease

- Costs
- Multiple scenarios
- Multiple therapeutic algorithms (“standard of care” not gold standard)
- No definitive evidence that one option is clearly better than others

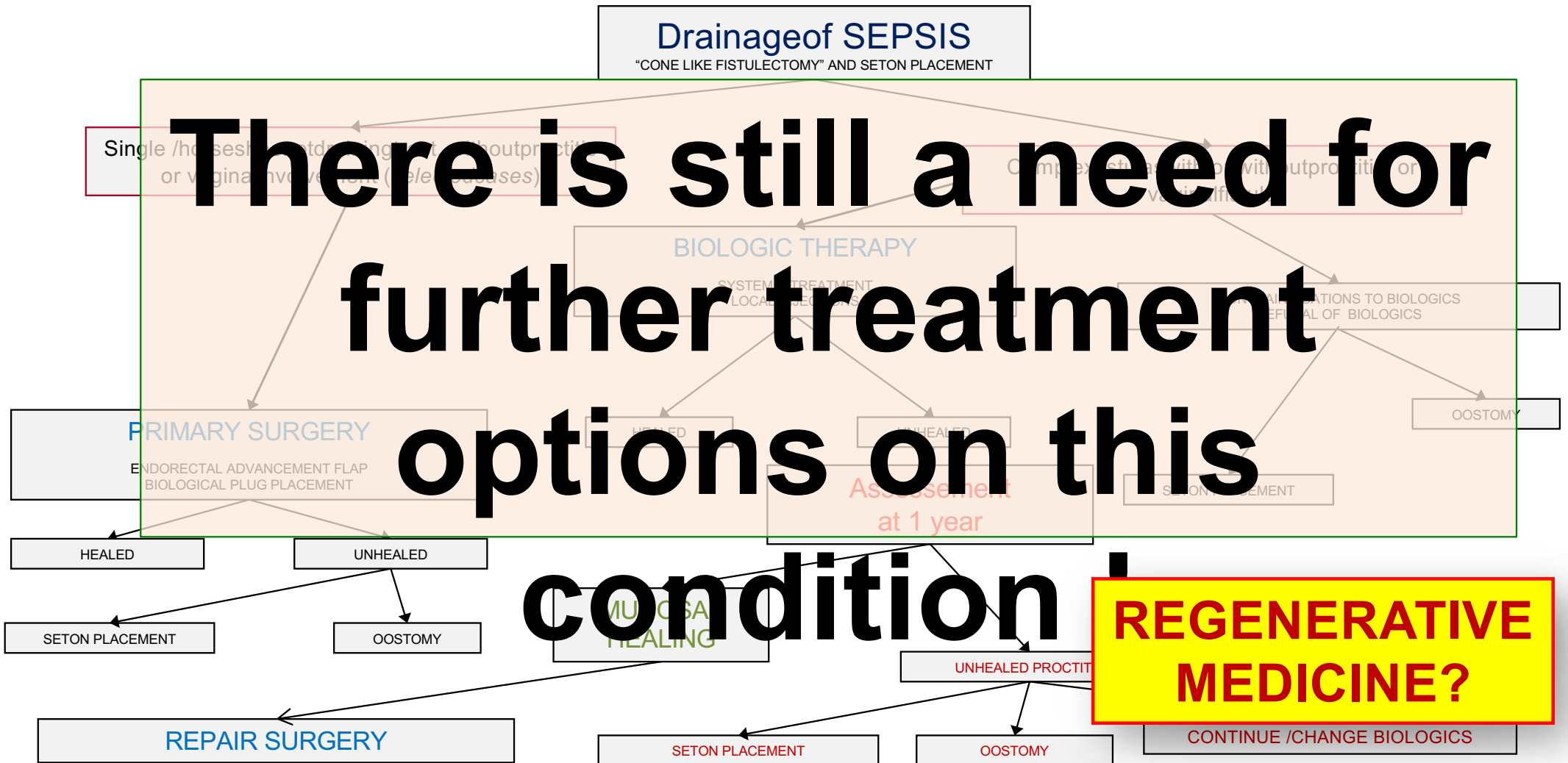
High rate of failure and relapses !!!

Panes et al, WJG 2018

Proportion of patients receiving types of treatment in a retrospective Dutch study (n = 181)²



Regenerative Medicine for Immune-mediated Diseases (IMIDIs)



La Medicina Rigenerativa

The Body Has Limited Ability to Regenerate



Humans can naturally regenerate some tissues, including the liver.

Unfortunately, many other human tissues do not regenerate.

La Medicina Rigenerativa

Campo medico che si occupa della

- ✓ **Riparazione**
- ✓ **Rigenerazione**
- ✓ **Sostituzione**

di tessuti o organi danneggiati o malati.

Ciò avviene attraverso l'uso di terapie cellulari specifiche, utilizzo di biomateriali di ultima generazione e ingegneria tissutale.

“Supererò le correnti gravitazionali, lo spazio e la luce per non farti invecchiare”

Se potessimo *rigenerare le cellule, i tessuti e gli organi* del nostro corpo a mano a mano che si corrompono, se potessimo *riprogrammare le cellule come se fossero un software*, se potessimo *fermare l’invecchiamento* dell’intero organismo, potremmo **prolungare la nostra vita illimitatamente**.

Quello **dell’elisir dell’eterna giovinezza** è uno dei sogni più antichi dell’uomo.



“This is not the end. It is not even the beginning of the end. But it is, perhaps, **the end of the beginning**”

W Churchill, 10 November 1942

“Supererò le correnti gravitazionali, lo spazio e la luce per non farti invecchiare”

Il vecchio sogno degli alchimisti si è ripresentato all'alba del nuovo millennio grazie agli sviluppi della **medicina rigenerativa** e, in particolare, delle terapie a base di cellule staminali

Nuova pietra filosofale  terapeutica.

clonazione

The New Daily Times

*“Somewhere between 10 and 20 years from now, there is going to be **tremendous transformation of health and medicine**. By treating biology as software and reprogramming cells to treat diseases and other ailments, humans have already made tremendous progress in medicine. These will be 1,000 times more powerful by the end of the decade. And a million times more powerful in 20 years”*

Ray Kurzweil, @ “Global Futures 2045”, 16 giugno 2013,

*“Sposta la bottiglia e lasciarmi guardare
Se di tanti capelli ci si puo’ fidare”*

The “PIXIE DUST”

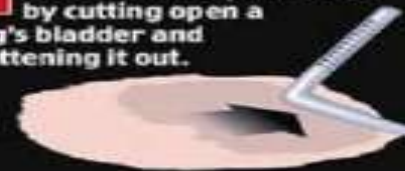
Regenerative Medicine In the Media



**“Pixie Dust”
Ground pig small intestinal
submucosa (SIS) from
Cook Biotech**

HOW TO REGROW A SEVERED FINGER

1 Start to make 'pixie dust' by cutting open a pig's bladder and flattening it out.



2 Scrape away the layer of muscle before 'cleaning' the remaining collagen-rich tissue by shaking it in acid.

3 Dry out the paper-like 'extra-cellular matrix' and grind into powder form.

4 Regularly sprinkle powder on severed finger tip.



5 Within a few weeks, tip grows to normal length, complete with nail and 'fingerprint'.



“I Russi, i Russi Gli Americani”

Nel 2014 Google ha fondato **Calico** (California Life Co), una società di biotecnologie che ha immediatamente annunciato un investimento di 250 milioni di dollari, con **l'obiettivo di sconfiggere l'invecchiamento**. Al progetto si è unito Il gigante farmaceutico AbbVie con un investimento di altri 250 milioni di dollari. L'investimento è aumentato fino ad arrivare alla stratosferica cifra di **un miliardo e mezzo di dollari**.



Fermare la malattia e la morte con la medicina rigenerativa non è più soltanto il sogno di tecnofili visionari ma il progetto di potenti multinazionali.

Cell-based products

All those treatments whose effects rely on the presence of cells

WHAT?

- Tissue-specific cells

- Mature cells
- Progenitor cells

- Mesenchymal stem cells

- Adipose tissue
- Bone marrow
- Fetal annexes (cord blood, amnion, placenta) → allogenic therapy

- Monocytes/macrophages (PBMNCs)

HOW?

- In vitro expanded

- ATMPs (complex regulatory pathways)
- Homogeneous cell population/characterized
- Costly (>15.000 €/procedure)



- Prepared at the point-of-care (POC)

- Minimally manipulated (less complex regulatory pathways)
- Heterogeneous cell populations/non-characterized
- More affordable



*rubatele pure i soldi rubatele anche i ricordi
ma lasciatele sempre la sua dolce curiosità*

late 1970s: Professor Marshall Urist, (Orthopedic UCLA) : effects of demineralized bone and the formation of de novo new bone in the muscle pouch of adult mice.



morphogenetic proteins, or BMPs

1991: Arnold Caplan (Case Western, Cleveland OH): receptive cells in the adult mouse muscle that responded to the BMPs.



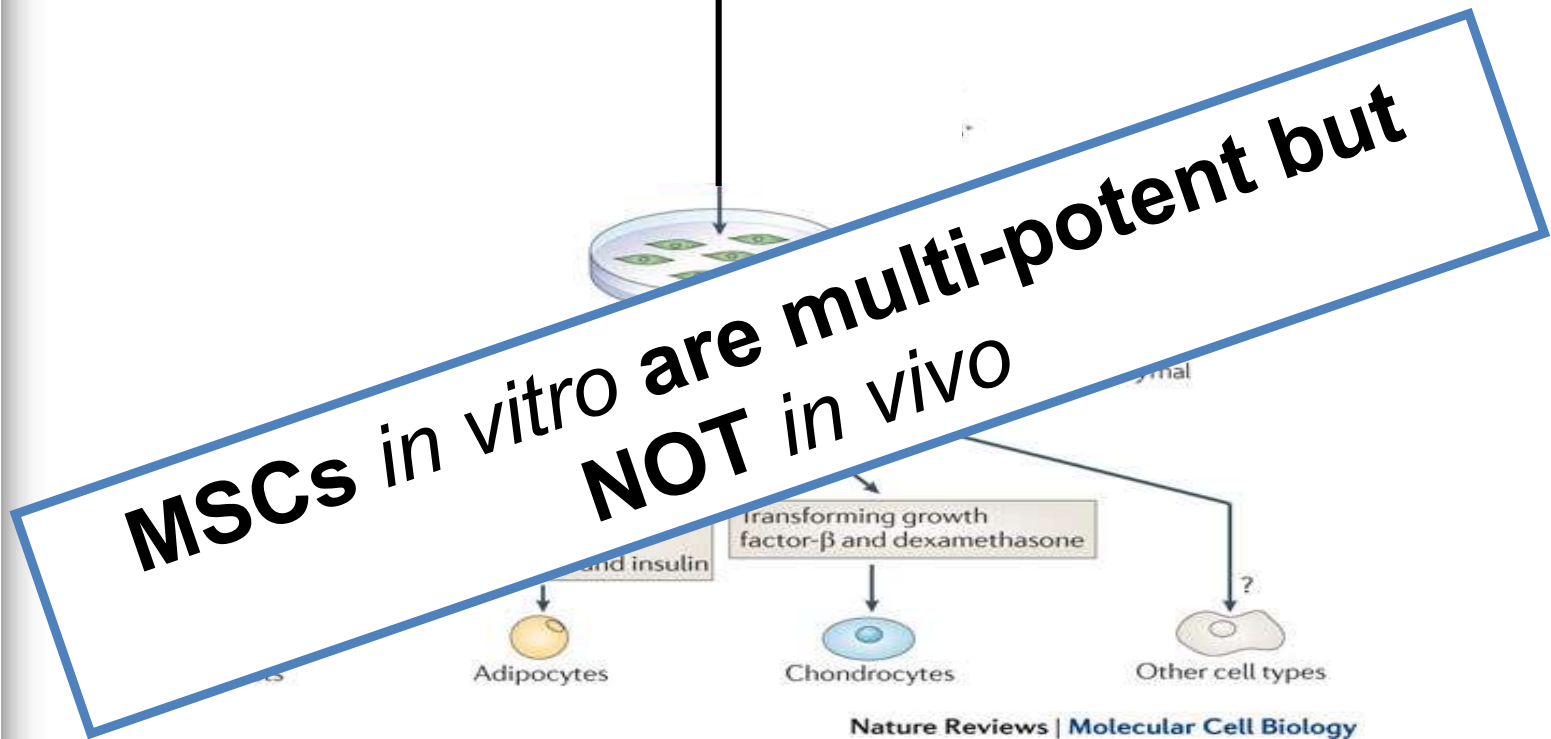
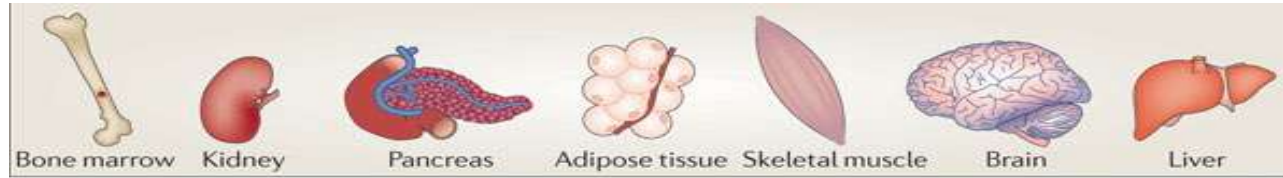
adherent human marrow cells **mesenchymal stem cells (MSCs)**,



multipotent cells

MSCs can be derived from multiple tissue sources

- Aorta
- Adipose
- Amniotic fluid
- Bone marrow
- Blood
- Brain
- Cartilage
- Cord blood
- Dental pulp
- Endometrium
- Eye
- Gut
- Heart
- Kidney
- Liver
- Lung
- Muscle
- Pancreas
- Perichondrium
- Periodontal ligament
- Placenta
- Salivary gland
- Skin
- Spleen
- Synovial membrane
- Tendon
- Thymus
- Umbilical cord
- Vein

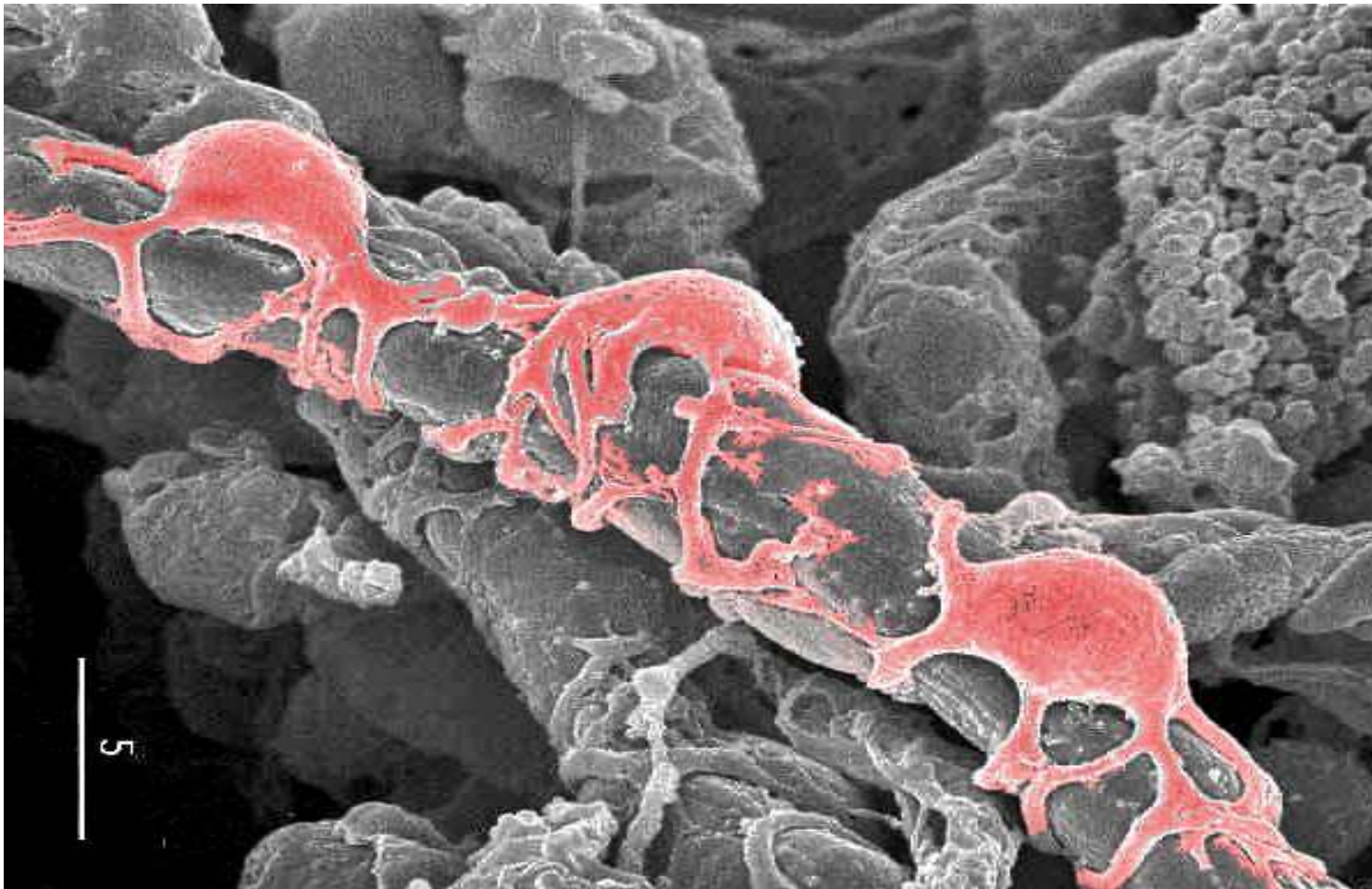


Murray et al, 2012

Nombela-Arrieta et al., 2011

Pericytes: cells on capillaries and microvessels.

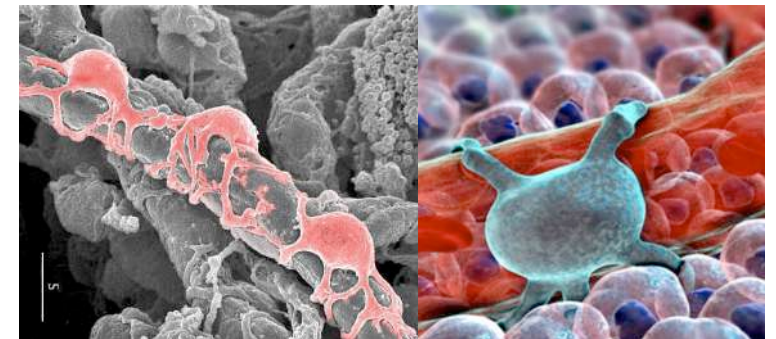
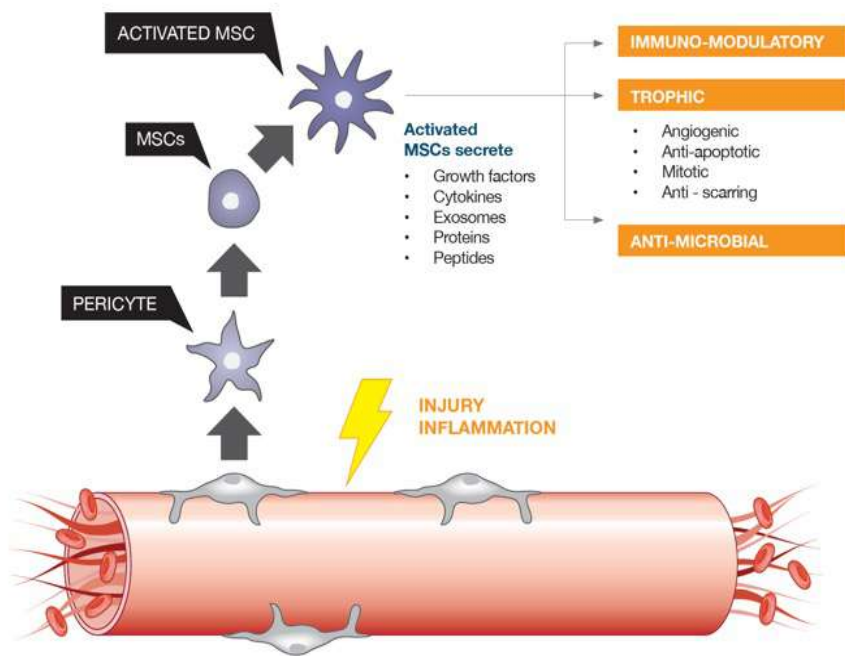
ALL Mesenchymal Stem Cells are PERICYTES!



Caplan, 2012

MSCs sense and interact with the local environment

MSCs possess the ability to **sense** the environment and **secrete** a plethora of trophic and immunomodulatory molecules

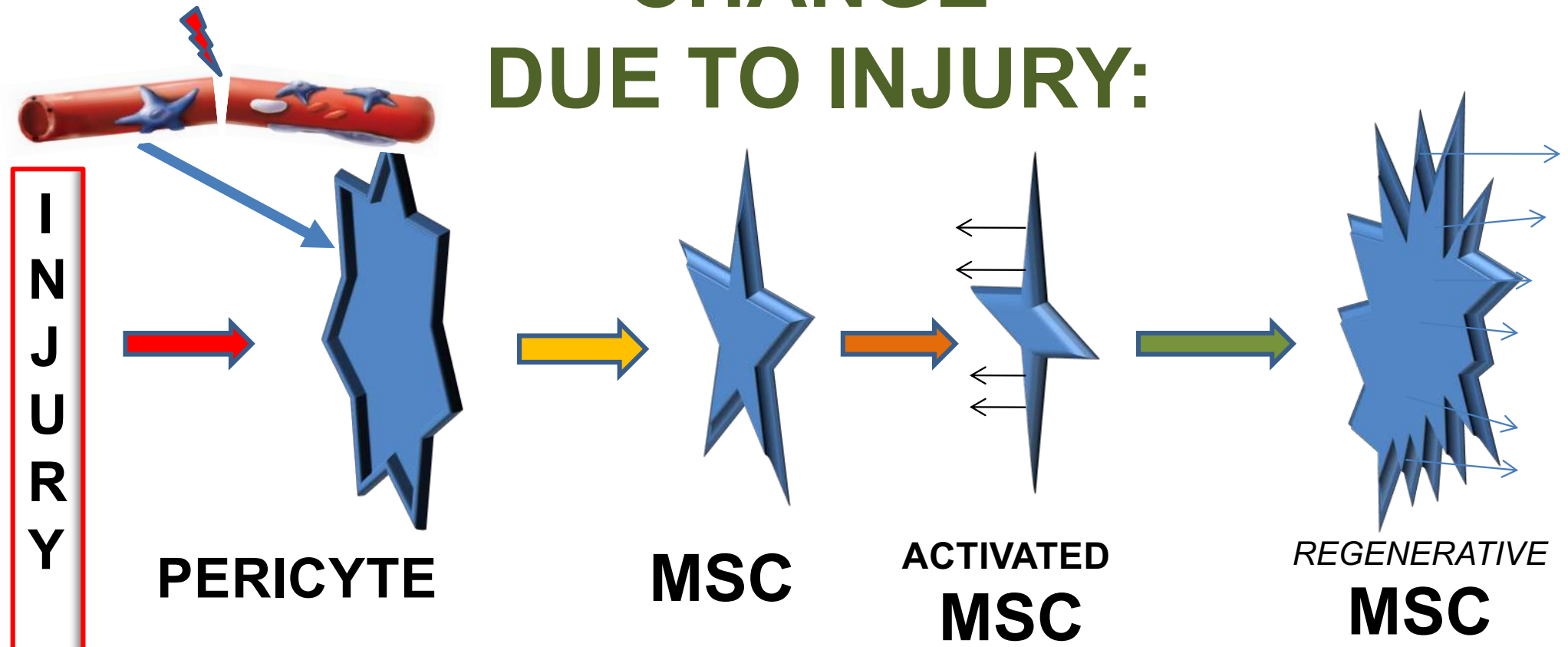


CD146⁺CD34⁻CD45⁻ cell population

2012

PROPOSED SEQUENCE OF CHANGE

DUE TO INJURY:



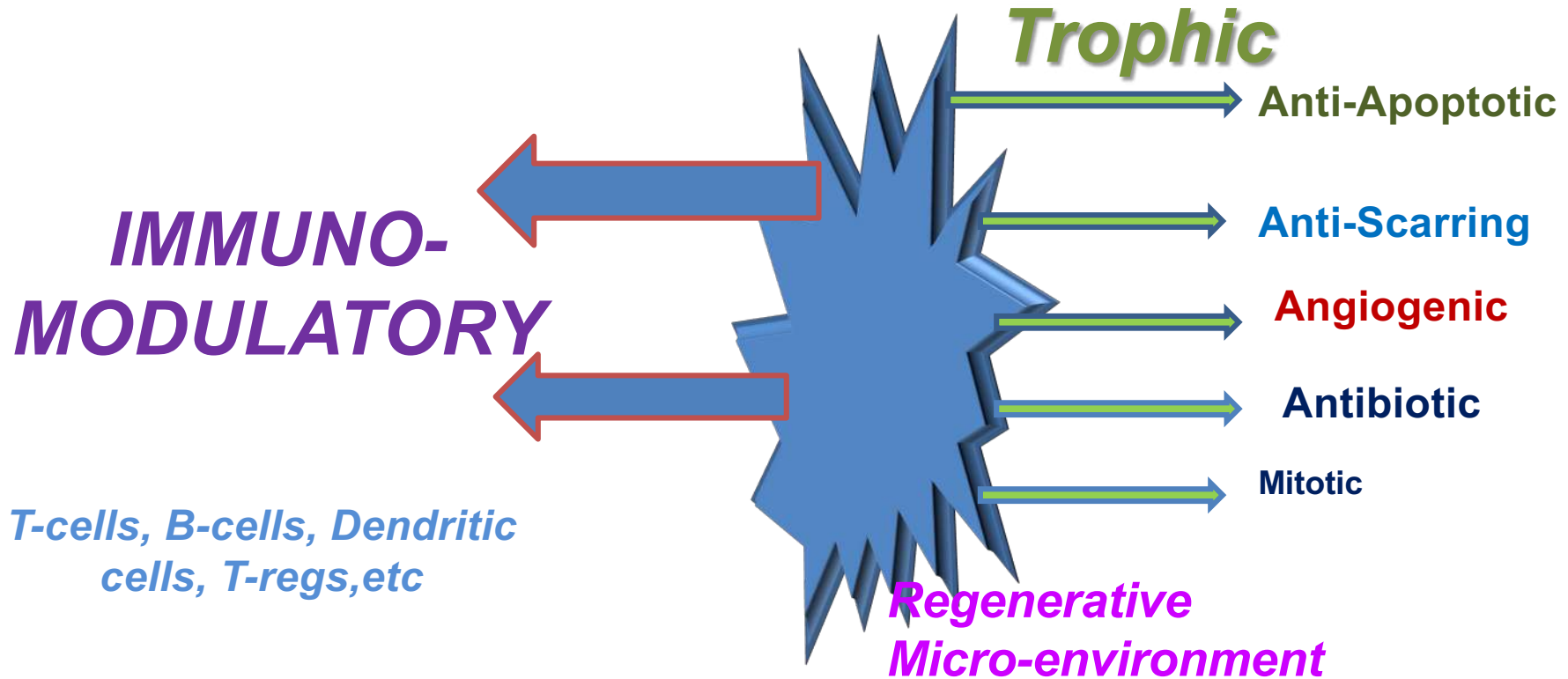
Al Caplan. MSCs as Therapeutics. In: *Stem Cell Biology and Regenerative Medicine. Mesenchymal Stromal Cells: Biology and Clinical Applications, Stem Cell Biology and Regenerative Medicine*. P. Hematti and A. Keating (Eds.), Springer Science+Business Media New York, Chapter 5. Pp.79-90, 2012.

MSC = *Medicinal Signaling Cell.*

(the injury-specific DRUG STORE)

2012

natural **INJURY RESPONSE**



MSC = *pericyte*

The "Vacanti Mouse" - 1997



Cao et al., *Plast Reconstr Surg*, 100: 297-302, 1997



THE NEW ERA OF REGENERATIVE MEDICINE

Dozens of biotech companies and university labs are developing ways to replace or regenerate failed body parts. Here are a few of the projects:



BONE

Bone-growth factors or stem cells are inserted into a porous material cut to a specific shape, creating new jaws or limbs. A product that creates shinbones is in clinical trials.

COMPANIES: Creative Biomolecules, Orquest, Sulzer Orthopedics Biologics, Genetics Institute, Osiris Therapeutics, Regeneration.



SKIN

Organogenesis' Apligraf, a human-skin equivalent, is the first engineered body part to win FDA approval, initially for leg ulcers. Other skins are in the works for foot ulcers and burns.

COMPANIES: Organogenesis, Advanced Tissue Sciences, Integra LifeSciences, LifeCell, Ortec International.



PANCREAS

Insulin-manufacturing cells are harvested from pigs, encapsulated in membranes, and injected into the abdomen. The method has been tested in animals and could be in human trials in two years.

COMPANIES: BioHybrid Technologies, Neocrin, Circe Biomedical



HEART VALVES, ARTERIES, AND VEINS

A 10-year initiative to build a heart has just started. Genetically engineered proteins have been successfully used to regrow blood vessels.

COMPANIES: Organogenesis, Advanced Tissue Sciences, Genetech, LifeCell, Regeneration.

DATA: BUSINESS WEEK, DRUG & MARKET DEVELOPMENT REPORTS



SALIVA GLANDS

Proteins called aquaporins that allow cells to secrete water are used to recreate saliva glands damaged by disease or radiation. Glands are also being engineered to secrete healing drugs. The technique has proven successful in mice.

COMPANIES: None yet.



URINARY TRACT

Cartilage cells are taken from the patient, packed into a tiny matrix, and injected into the weakened ureter, where they bulk up the tissue walls to prevent urinary backup and incontinence. The method is in late-phase clinical trials.

COMPANIES: Regeneration, Integra LifeSciences.



BLADDER

Doctors at Children's Hospital in Boston have grown bladders from skin cells and implanted them in sheep. They are about to try the same process on a patient.

COMPANIES: Regeneration.



CARTILAGE

A product is already on the market that regrows knee cartilage. A chest has been grown for a boy and a human ear on a mouse.

COMPANIES: Genzyme Tissue, Biomatrix, Integra LifeSciences, Advanced Tissue Sciences, ReGen Biologics, Osiris Therapeutics.



TEETH

Enamel matrix proteins are used to fill cavities. It works in dogs; human trials are a few years away.

COMPANIES: Biora, Atrix Laboratories, Creative BioMolecules.



BREAST

In preclinical studies, several companies have been able to create a cosmetic nipple by inserting a ball of cartilage. Researchers are now trying to grow a whole cosmetic breast.

COMPANIES: Regeneration, Integra LifeSciences.



LIVER

A spongy membrane is built up and then seeded with liver cells. Organs the size of a dime have been grown, but a full-size liver could take 10 years due to its complexity.

COMPANIES: Advanced Tissue Sciences, Human Organ Sciences, Organogenesis.



SPINAL CORD NERVES

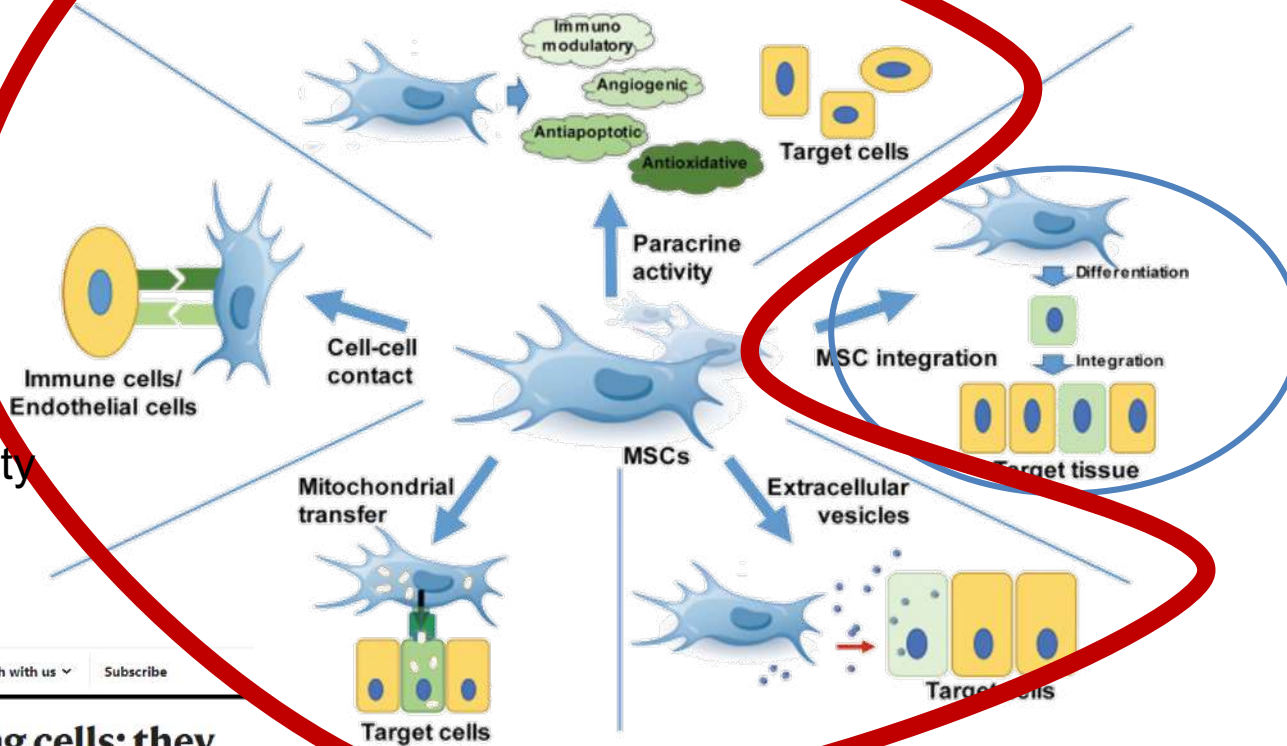
Scientists are investigating nerve-growth factors, injecting them at the site of damage to encourage regeneration or seeding them along biodegradable filaments and implanting them. Rats have been made to walk again.

COMPANIES: Acorda, Regeneration, CytoTherapeutics, Guilford Pharmaceuticals.

Mechanism of action of MSCs



Paracrine ability



Differentiation ability

nature

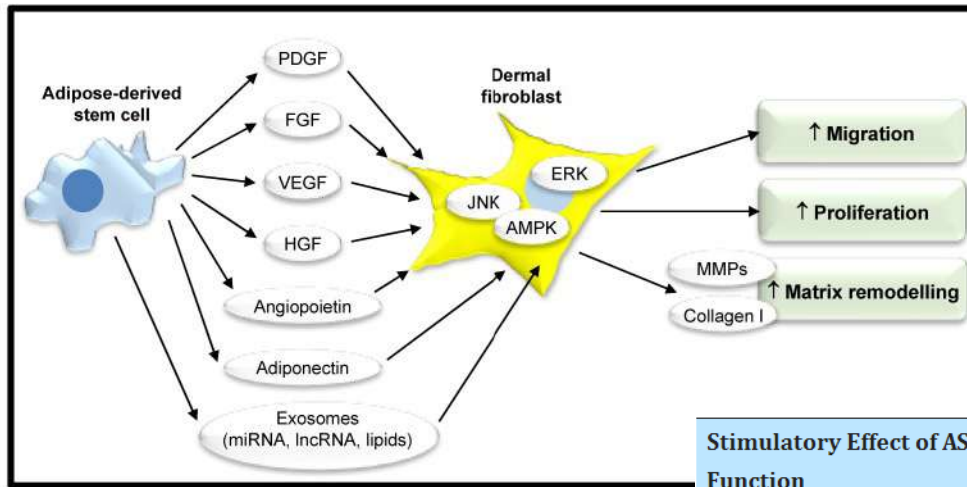
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Medicinal signalling cells: they work, so use them

Arnold J. Caplan

MSC= Medicinal Signaling Stem Cells

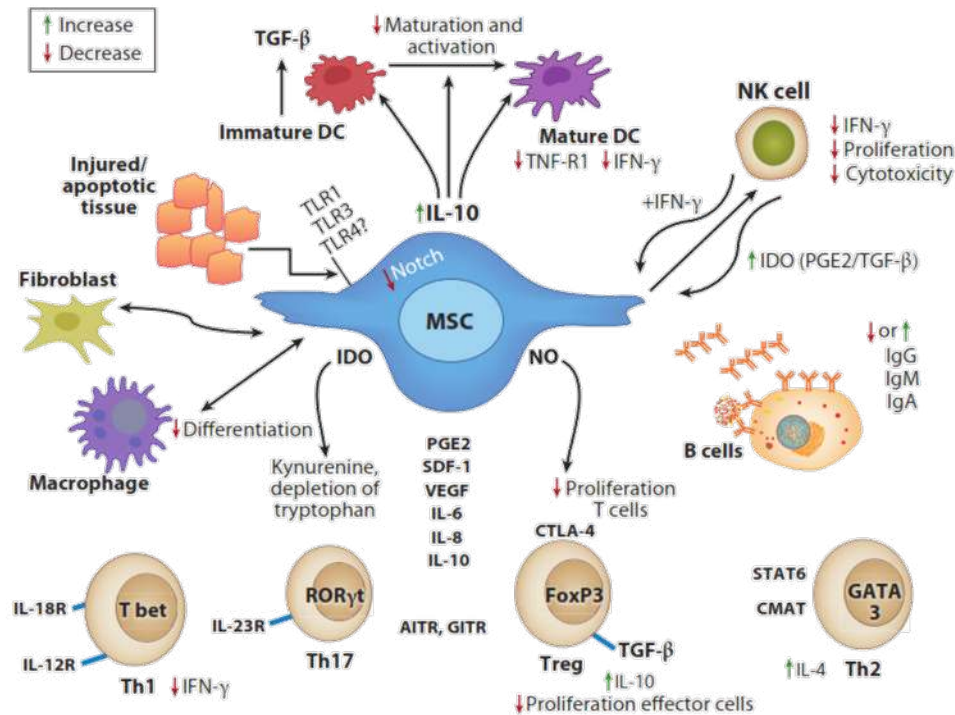
ASCs effects on dermal fibroblasts



Stimulatory Effect of ASC Secretome on Dermal Fibroblast Function	Paracrine Factors Secreted by ASCs	References
Increased proliferation	FGF-2	[45,46,60]
	HGF	[60]
	Exosomes	[56,61]
	Microvesicles	[57]
Increased migration	FGF-2	[45,46]
	Exosomes	[45,56,61]
	Microvesicles	[57]
Matrix Remodeling (increased production of collagen I and III, TGFβ, FGF2, and MMP1)	Adiponectin	[49]
	Exosomes	[56,61]

Trevor L. et al. Journal of Clinical Medicine 2020

MSCs/pericytes modulate immune system



- ↓ Pro-inflammatory cytokines
- ↑ Anti-inflammatory cytokines
- ↓ NK cells
- ↓ T-Cells
- ↓ Dendritic cells

MSCs actively participate to the healing process of a tissue through the interaction with resident cells

- ✓ Terminating inflammation
- ✓ Creating a more receptive microenvironment for the action of reparative stem cells

Cell-based products for regenerative medicine: Why Adipose Tissue?

- Minimally invasive harvest
- Frequency of **MSCs** in adipose tissue is very high (50-500 times higher than in bone marrow)
- Poorly immunogenic (not immuno-privileged ...)
- Pronounced anti-inflammatory and immunomodulatory effect
- Not strictly age-dependent (even if affected by several donors' features)



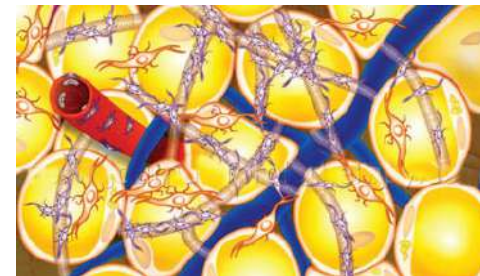
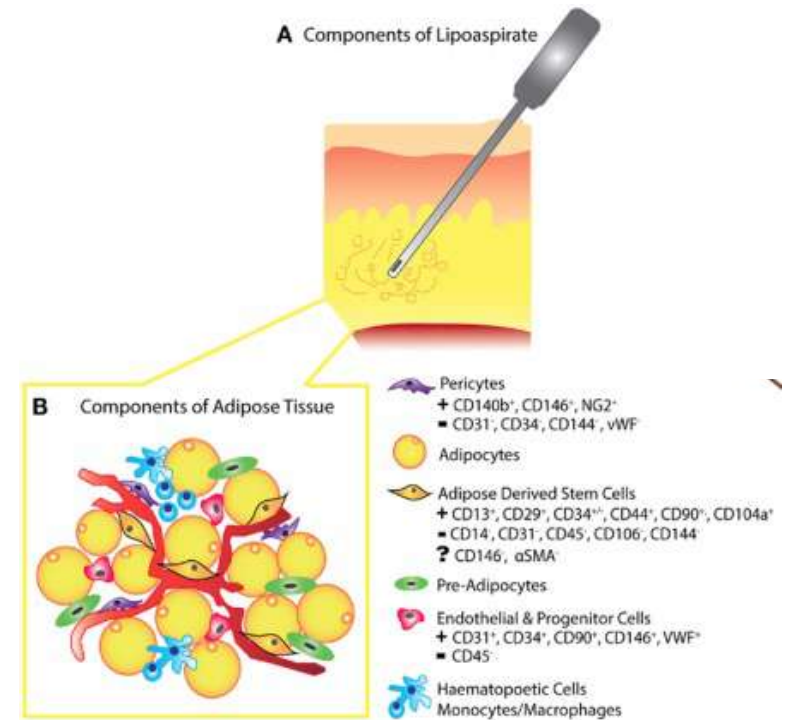
Zuk PA, *Tissue Engineering* 2001
De Ugarte DA, *Cell Tissue Organs* 2003
Guilak F, *J Cell Physiology* 2006
de Girolamo et al., *Cytotherapy* 2009
Ceccarelli S et al 2020

Adipose tissue - *Composition*

- Adipocytes
- **MSCs/Pericytes** (wrapped around capillaries)
- Pre-adipocytes (progenitor cells)
- Other cell types

- Extracellular matrix (collagen and connective tissue)
- Microvasculature

MSCs are embedded in a niche



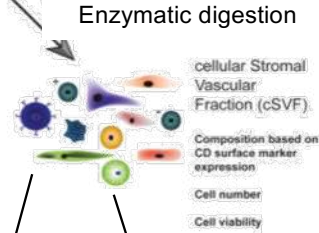
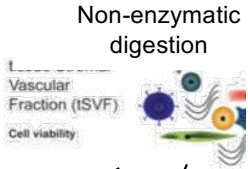
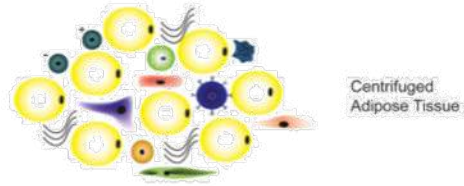
Adipose tissue cell-based products



**OFFICE/
R**



Mechanical SVF
Microfragmented FaSVF



AS
Cs



LABORATORY

Legend	
	Adipocyte
	Extracellular matrix
Adipose Derived	
	Progenitor pericyte CD31 ⁺ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ⁻
	Pericyte CD31 ⁻ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ⁻
	Supra-adventitial cell CD31 ⁻ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ⁻
	Vascular endothelial cell CD31 ⁺ CD34 ⁺ CD45 ⁺ CD90 ⁻ CD105 ⁻
	Fibroblast CD31 ⁻ CD34 ⁻ CD45 ⁻ CD90 ⁻ CD105 ⁺
	Adipose Derived Stromal Cell CD31 ⁻ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ⁺
	Endothelial cell CD31 ⁺ CD34 ⁺ CD45 ⁺ CD90 ⁻ CD105 ⁻
Blood Derived	
	Granulocyte
	Monocyte/macrophage
	Lymphocyte
	Hematopoietic stem cell
Culture Derived	
	Culture derived Adipose Stromal Cell CD31 ⁻ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ⁺

Modified from Van Dongen et al., J TERM 2018

FLAWS OF MSCs CELL THERAPIES

Despite the encouraging results, utilization in daily clinical practice is limited by:

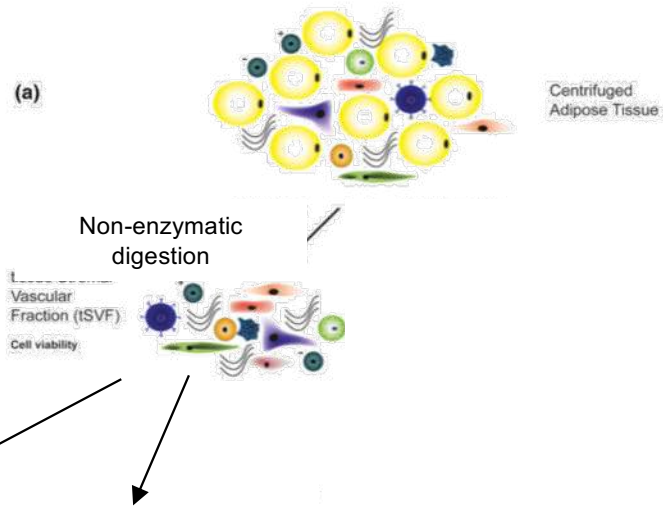
- ✓ Need of GMP grade (good manufacturing practice) laboratories
- ✓ Time interval (several weeks) required for *in vitro* cell expansion, which represents a costly and time-consuming approach
- ✓ Complex restrictions related to cell expansion and extensive manipulation.

FLAWS OF MSCs CELL THERAPIES

These limitations spurs the need to develop **novel regenerative approaches** to isolate autologous adipose tissue containing MSCs, which should possibly be

- ✓ “one-step”,
- ✓ minimally invasive,
- ✓ not requiring any enzymatic treatment,
- ✓ affordable
- ✓ compliant with the regulatory panorama..

Adipose tissue cell-based products



Legend	
	Adipocyte
	Extracellular matrix
Adipose Derived	
	Progenitor pericyte CD31 ⁻ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ⁻
	Pericyte CD31 ⁻ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ⁻
	Supra-adventitial cell CD31 ⁻ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ⁻
	Vascular endothelial cell CD31 ⁺ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ^{int}
	Fibroblast CD31 ⁻ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ⁻
	Adipose Derived Stromal Cell CD31 ⁻ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ^{int}
	Endothelial cell CD31 ^{int} CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ^{int}
Blood Derived	
	Granulocyte
	Monocyte/macrophage
	Lymphocyte
	Hematopoietic stem cell
Culture Derived	
	Culture derived Adipose Stromal Cell CD31 ⁻ CD34 ⁺ CD45 ⁻ CD90 ⁺ CD105 ⁺



OFFICE/OR



Mechanical Microfragmented Fat
SVF

Modified from Van Dongen et al., J TERM 2018



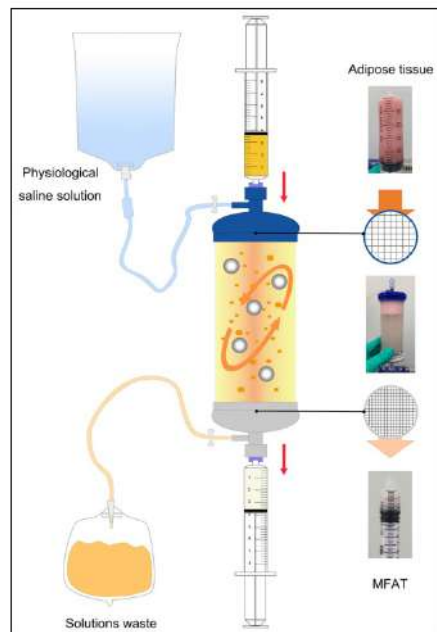
Not all the same!!!

*Sei così bella.. Anzi
bellissimissima*

Why LIPOGEMS ?

- Lipogems® is the **most studied product among those based on the minimally manipulation of adipose tissue** → Fundamental for results
- The mechanisms of action of Lipogems have been **clearly demonstrated in a variety of in vitro models**, confirming the maintenance of the stem cell niche
- Its anti-inflammatory role is reported in most of the investigations, both in vitro and **preclinical/clinical**

Features of microfragmented adipose tissue with Lipogems

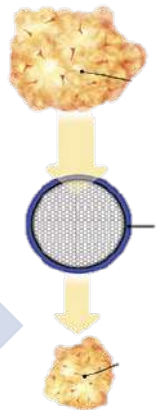


Washes & Rinses Normal saline removes pro-inflammatory impurities such as OIL and BLOOD



Resizes Micro-fragments the adipose tissue - OPTIMAL VISCOSITY/HANDLING

Preserves microarchitecture /niche Preserves the cell and tissue microarchitecture protecting pericytes and strengthening their effectiveness in the recipient environment



AUTOLOGOUS MICROFRAGMENTED ADIPOSE TISSUE (TAAM) INJECTION

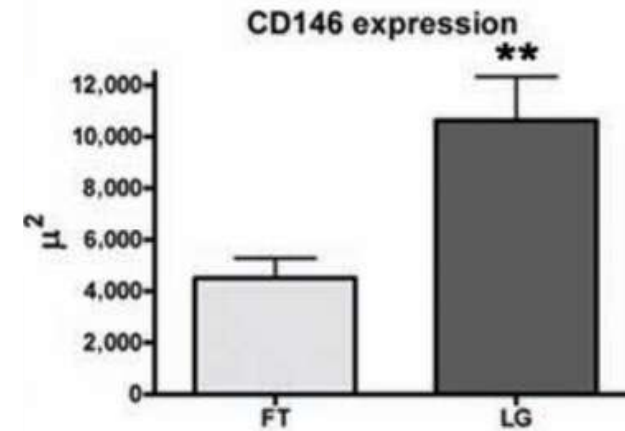
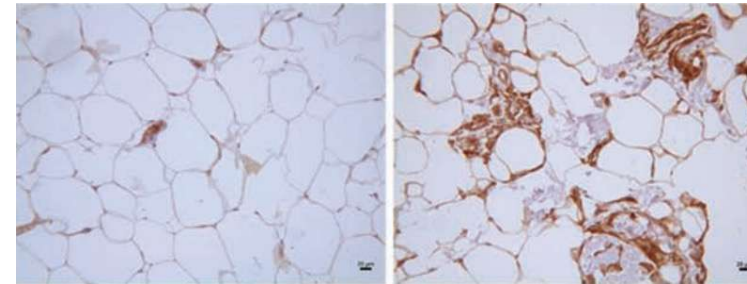
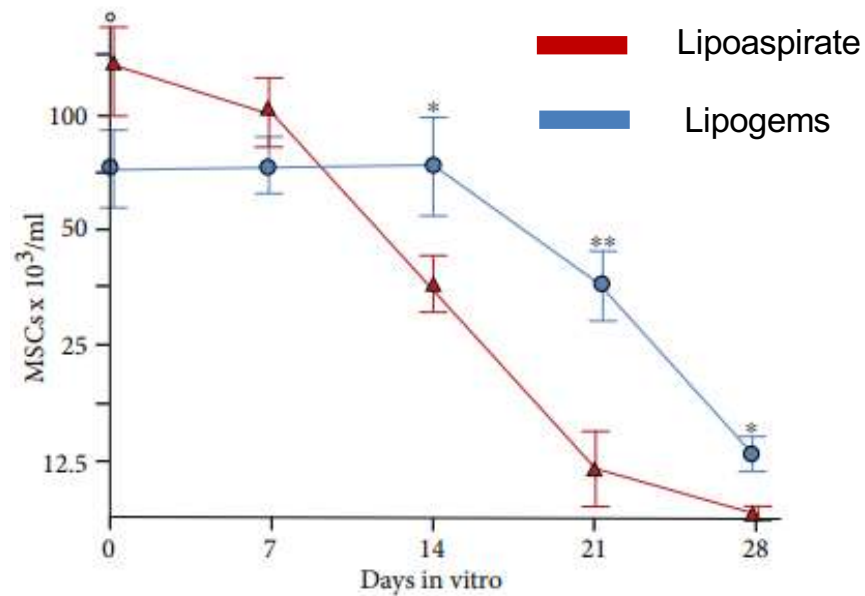
LIPOGEMS - TAAM



The Lipogems product represents a **biodynamic filler containing intact vasculo-stromal niches rich in pericytes**, without any loss of the architecture and vitality of its cellular elements. The purpose of the **pericyte** is **not** that of **transdifferentiation**, which is absent, but is to **promote a microenvironment that allows the natural inclination of the receiving tissue to self-heal** to emerge.

The pericytes and the cells derived from them therefore do not represent elements capable of assuming phenotypic orientations different from those of the starting tissues, but they actually become **facilitators of a self-healing process**

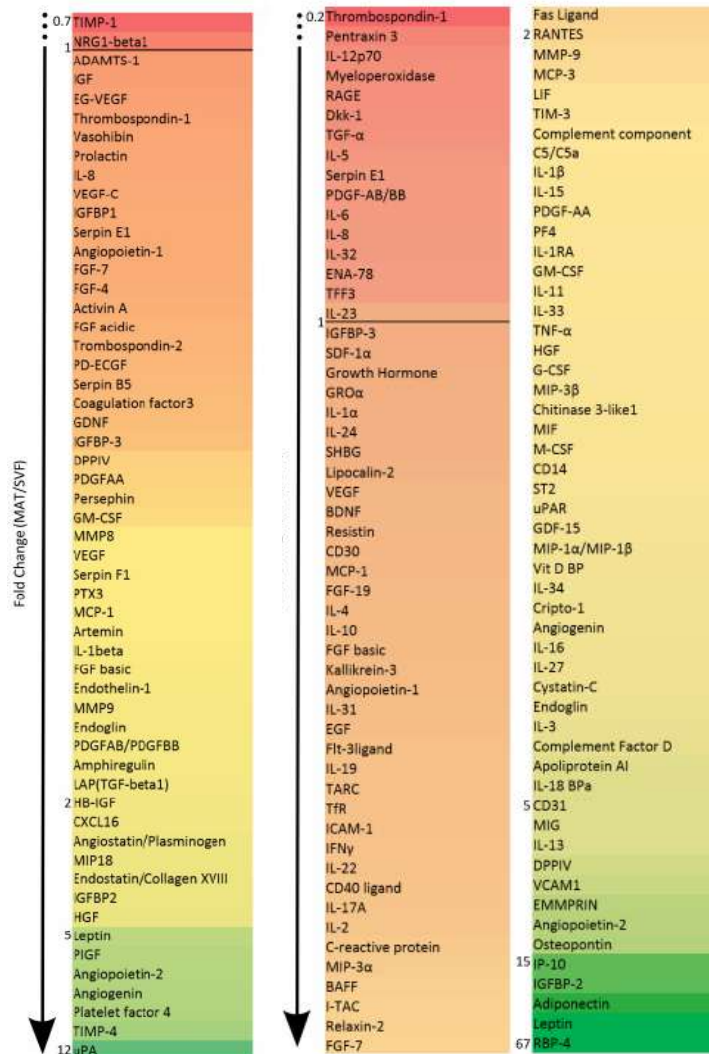
The importance of the stem cell niche



Nava S et al., *Stem Cells International* 2019

Bianchi F et al., *Cell Transplantation* 2013

The importance of the stem cell niche



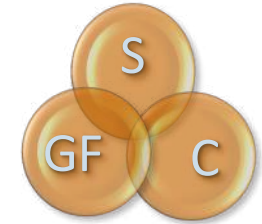
Microfat produces higher amounts of many tested growth factors and cytokines, as compared with enzymatically dissociated cells

- Adiponectin (metabolic cascades)
- RBP-4 (retinol binding protein)
- IL-1RA (antiinflammatory)
- Leptin (regulating diverse mediators of immunoinflammation, leukocyte recruitment, and migration)
- GDF-15 (regulator of inflammation) MIF (controls inflammation and innate immunity)
- MIG-CXCL9 (T-cell chemoattractant)
- MIP3β-CCL19 (immune cell migration)
- RANTES- CCL5 (chemotactic for multiple leukocytes)

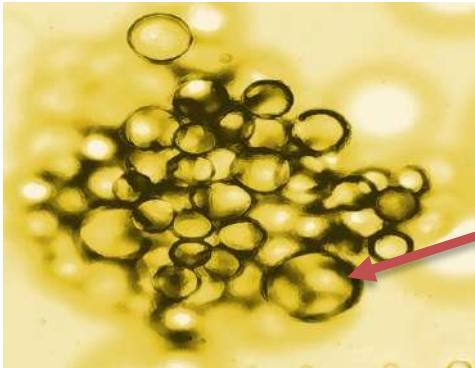
Vezzani et al., Stem Cells Translational Medicine 2018



It is the BEST LIPOASPIRATE PREPARATION
(natural bioreactor)



- Lipogems is a smooth and injectable **tissue** with optimal viscosity for a small gauge needle (20-22g) and it is easy to **engraft** in recipient tissue working for a long time



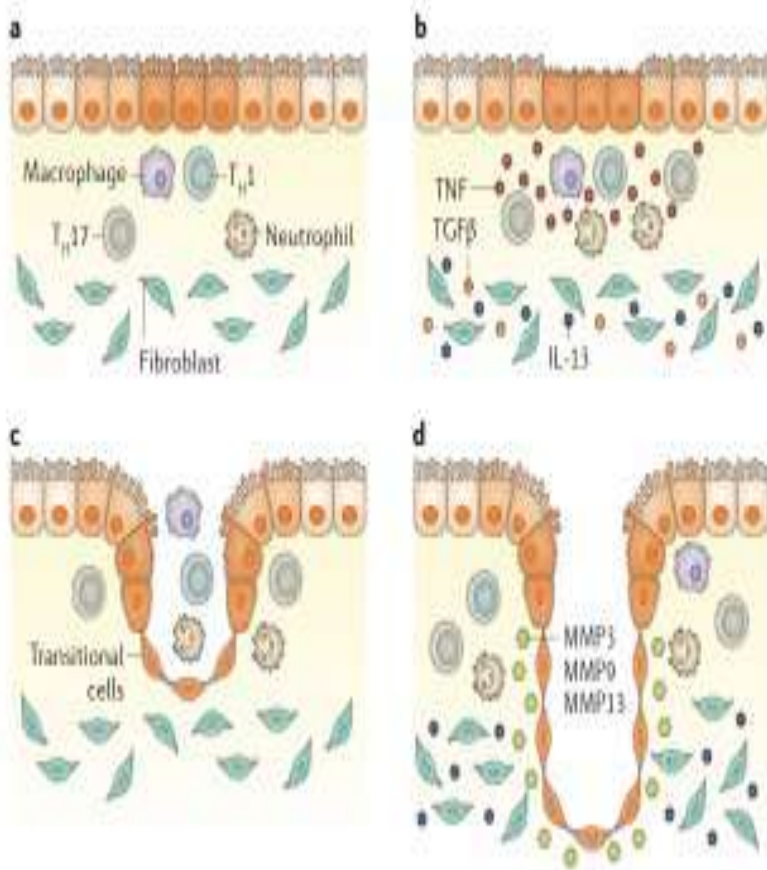
- 100% Live tissue
- IDEAL SIZE adipose cluster for GRAFTING with **preserved cell and tissue microarchitecture** that contains the structural components and microvasculature including pericytes, adipocytes, and other cells that are essential to help to facilitate a healing microenvironment.
- Reduced quantity impurities that cause inflammation (blood and oil)



**LONG TERM ENGRAFTMENT IN DIFFICULT
TISSUES** (synovia, vaginal mucosa, scars)

Regenerative Medicine for Immune-mediated Diseases (*IMIDIs*)

Rationale for use in **perianal CD**



IMMUNOMODULATORY ACTION

Dampen the inflammatory response:

- reducing activation of CD4 T lymphocytes
- promote the formation of regulatory T-cells

Dampen the proinflammatory action of dendritic cells

Anti inflammatory action on Bcells reducing the activation and production of antibodies and reducing the toxicity of natural killers cells

REGENERATIVE CAPABILITY

i.e. can transform into fibroblasts to help form a scar...

Review Article

Efficacy and Safety of Mesenchymal Stem Cells in Treatment of Complex Perianal Fistulas: A Meta-Analysis

Fang Cheng, Zhong Huang, and Zhi Li

Stem Cells International, 2020

7 RCTs (out of 1080 studies !) with 730 pts

Risk of Bias

Study	Type of perianal fistula	N	Cell type and source	Outcome	Results (healed)
Garcia-Olmo et al. [14] (2009) Spain	(1) Crohn (2) Cryptoglandular	49	Autologous ASCs	Reepithelialization	17/24 vs. 4/25 CD; 5/7 MSCs+fibrin glue vs. 1/7 fibrin glue at 8 w; cryptoglandular: 12/17 MSCs+fibrin glue vs. 3/18 fibrin glue at 8 w;
Guadalajara et al. [16] (2012) Spain	(1) Crohn (2) Non-CD	30	Autologous ASCs	Reepithelialization+MRI	10/18 vs. 3/12 CD; 2/4 MSCs+fibrin glue vs. 1/2 fibrin glue at 40 w;
Herreros et al. [17] (2012) Spain	Cryptoglandular	183	Autologous ASCs	Reepithelialization+MRI	51/124 vs. 22/59 MSCs+26/60 MSCs+fibrin glue vs. 22/59 fibrin glue at 24 w
Molendijk et al. [22] (2015) Netherlands	Crohn	21	Allogeneic BMSCs	Reepithelialization+MRI	7/15 MSCs vs. 2/6 saline solution at 12 w
Panés J [23] (2016) Israel	Crohn	212	Allogeneic ASCs	Reepithelialization+MRI	53/103 MSCs vs. 36/101 saline solution at 24 w
Panés J [26] (2018) Israel	Crohn	212	Allogeneic ASCs	Reepithelialization+MRI	58/103 MSCs vs. 39/101 saline solution in 52 w
Garcia-Arranz M [27] (2019) Spain	Cryptoglandular	35	Autologous ASCs	Reepithelialization	10/20 MSCs+fibrin glue vs. 5/19 fibrin glue at 2 years



Using “**reepithelialization**”: **61.4%** versus 20.5%; (OR = 5.92; P = 0.02)

Using **MRI** (363 pts): **49.3%** versus 36.6% (OR = 1.77; P = 0.0006).

Refractory Complex Crohn's Perianal Fistulas: A Role for Autologous Microfragmented Adipose Tissue Injection

Silvio Laureti, MD, PhD,* Paolo Gionchetti, MD,† Alberta Cappelli, MD,‡ Laura Vittori, MD,* Federico Contedini, MD,§ Fernando Rizzello, MD, PhD,† Rita Golfieri, MD,‡ Massimo Campieri, MD,† and Gilberto Poggioli, MD*

IRCCS S. Orsola - University Hospital, Bologna - Italy

IBD, March 2020

15 pts with persistent complex fistulizing PCD after the bio-surgical approach and subsequent surgical “rescue” repair



After local surgical drainage of the perianal disease, 20 cc of micro-fragmented adipose tissue were injected circumferentially into the submucosa surrounding the internal fistula orifice
(CARRIED OUT IN DAY SURGERY SETTING !!!)

Clinical outcomes were determined at 24 week follow-up assessing success rate, defined as “*combined clinical and radiological remission*”
(absence of residual collections > 3 mm ..)

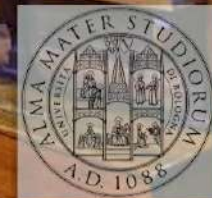
THE PROCEDURE



TRATTAMENTO DELLE FISTOLE PERIANALI COMPLESSE DI CROHN CON INOCULAZIONE LOCALE DI TESSUTO ADIPOSO AUTOLOGO MICROFRATTURATO

Silvio Laureti, Alberta Cappelli, Ferdinando Rizziello,
Federico Contedini, Laura Vittori, Paolo Gionchetti,
Maurizio Coscia, Lorenzo Gentilini, Gilberto Poggioli

Percorso Chirurgia del Tratto Alimentare
Dir Prof G. Poggioli
Università di Bologna



“REGENERATIVE” SURGERY

INTRAOPERATIVE PRECAUTIONS



For disinfection, **alcoholic solutions, hydrogen peroxide and povidone-iodine should be avoided** due to their cell toxicity, while non-alcoholic solutions, polyhexamethylene biguanide, octenidine dihydrochloride and chlorhexidine, can be used safely.

The molecules most frequently used for **local anesthesia and P.O analgesia** (of the amide type: ropivacaine, lidocaine, bupivacaine, mepivacaine) **should be avoided** due to a possible direct cytotoxic effect.

Setons must be removed

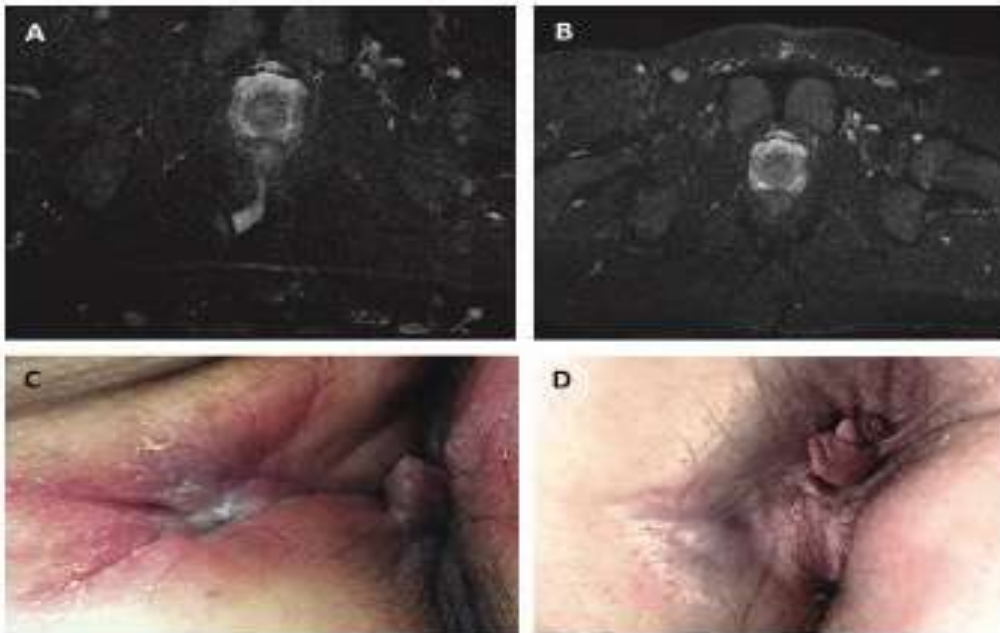
Refractory Complex Crohn's Perianal Fistulas: A Role for Autologous Microfragmented Adipose Tissue Injection

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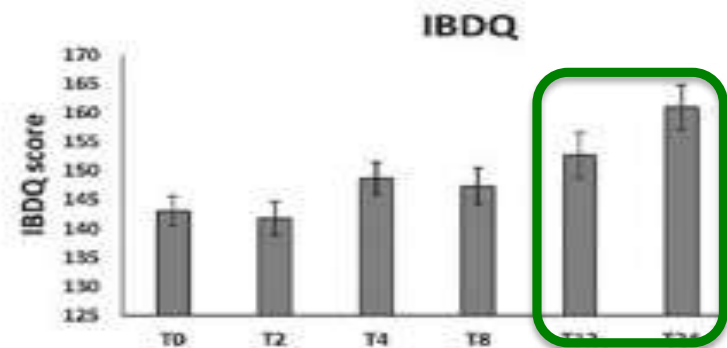
IBD, March 2020

RESULTS



10 / 15 pts (66.7%) with **COMBINED REMISSION** (healed)

14 / 15 pts (93.3%) with **CLINICAL REMISSION** (not draining fistulas at gentle finger compression)



Autologous Microfragmented Adipose Tissue Injection in Refractory Complex Crohn's Perianal Fistulas: a 5-year long-term update of the results (maintenance and rescue reinjection)

Laureti S, Torre B, Cappelli A, Salice M, Doussias N, Gionchetti P, Rizzello F, Poggioli G

(Submitted)

**FINAL SUCCESS RATE INCREASED TO 85.7 %
AFTER RESCUE RE-TREATMENT !!!!**

- ✓ **9** patients who reached combined remission at 24 weeks f.u. maintained healing
- ✓ **1** patient presented recurrence at 62 months was reoperated (lipogems) and healed
- ✓ **1** patient (failure group) refused reoperation due to paucity of symptoms with acceptable quality of life
- ✓ **4** patients (failure group) were **re-operated** ("Rescue Group")
 - ✓ **2** patients healed after Lipogems reinjection combined with mucosal flap
 - ✓ **1** patient with failure after Lipogems reinjection
 - ✓ **1** patient in f.u. after Lipogems reinjection and mucosal flap

Personal Experience (2016 – 2023)

118 procedures in **98** pts (10 with UC)
All pts refractory to multiple strategies
In **10** pts **reinjection**
In **1** pt 3rd injection



Crohn's
Rectovaginal
Pouch-anal
Pouch-vaginal
Sphincteroplasty

Results available on **89** pts with complete follow-up



Total success rate: **67 %**
Patient success rate: **77 %**

- ✓ In all cases but 1: fistulectomy (**1 recurrent abscess** !)
- ✓ In 87% of cases: associated surgical repair (*vertical or u-shaped flap, suture of the internal orifice, plug*)

Personal Experience (2016 – 2023)

In **10** pts: reinjection

In **1** pt: 3 injections

4 pts healed after first failure

2 pts unhealed after 2 injections

4 pts reinjected after healing for anal verge plasty (*minor incontinence*)

1 pt healed after **3** injections (*associated flap @ the last procedure*)



Multicentric randomized double blind prospective study **“ATTIC”** –

(Bologna IRCCS S. Orsola, Milano Sacco, Roma IRCCS Gemelli, Napoli Federico II, Firenze Careggi, Milano Humanitas) **Sponsored**

TREATMENT OF **MULTI-RESISTANT CROHN'S COMPLEX PERIANAL** FISTULA BY TRANSPLANTATION WITH LOCAL INFILTRATION OF MICROFRACTURED AUTOLOGOUS FAT TISSUE (LIPOGEMS) VS saline injection and suture @ internal orifice

Sample size: **80** pts

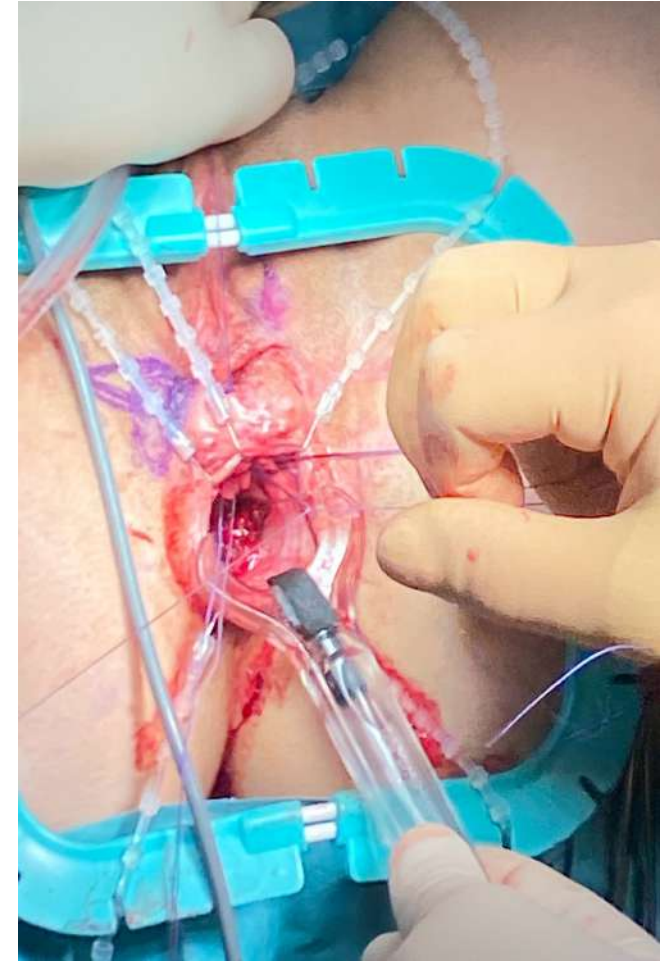


44 enrolled so far !

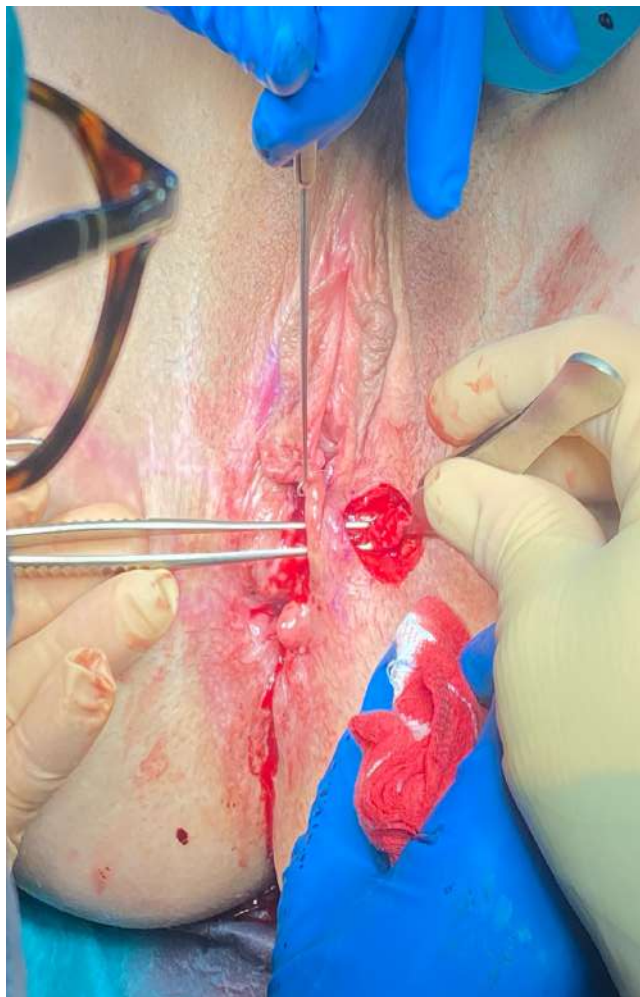
Monocentric randomized prospective study – **“LIPOPLUG”** - Grant Ricerca Corrente -

TREATMENT OF CROHN'S MULTI-RESISTANT RECTOVAGINAL FISTULAS BY COMBINATION OF TRANSPLANTATION WITH LOCAL INFILTRATION OF MICROFRACTURED AUTOLOGOUS ADIPOSE TISSUE (LIPOGEMS) AND PLACEMENT OF PLUGS IN COLLAGEN MATRIX (SURGIMEND)

LIPOPLUG



Anal verge plasty + Lipogems



OTHER INDICATIONS

ORTHOPEDICS

CLINICAL EVIDENCE (OA)



Procedures

In excess of 50,000 Lipogems procedures completed on OA human patients.



Clinical Trials

Over 35 published Clinical Trials. Large majority of independent self-funded studies.

- Knee
- Shoulder
- Other joints

9 ongoing Randomized Clinical Studies

- 6 in Knee OA, both for Standalone Injection and Surgical Adjoint protocols
- 3 in **Shoulder OA**



Safety

No major related adverse effect recorded.

- No sign of chondro-toxicity
- Vitality of chondrocytes, production of extracellular matrix proteins.



Efficacy

Results point to:

- **Lipogems standalone injection** as viable approach for the management of diffuse degenerative knee chondropathy in the **long-term (3+ years) for mild-moderate patients**

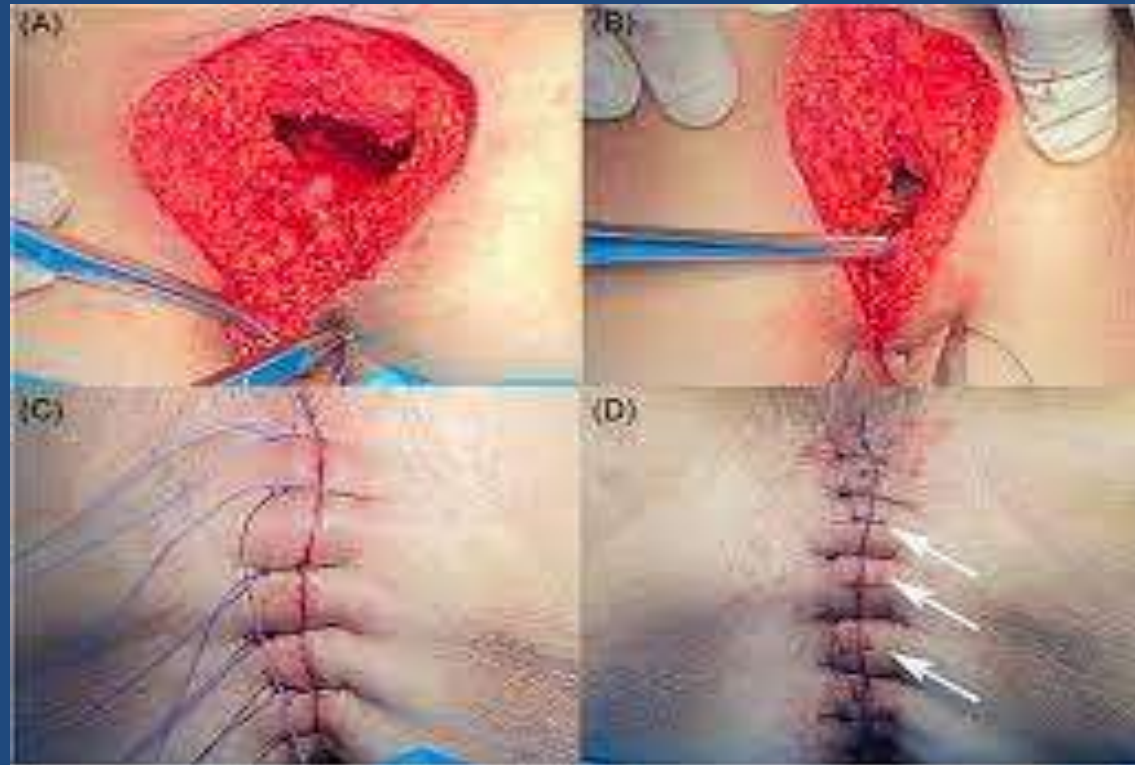
- **Lipogems** standalone injection and/or as an **arthroscopy adjoint** as a clinically effective and economically viable solution to **postpone TKR in severe patients**

- Lipogems injection as a safe and effective option for the **management of refractory shoulder pain** arising from shoulder conditions (Such as Glenohumeral Joint Arthritis or Rotator Cuff Pathology) in patients who failed previous conservative therapies

COLOPROCTOLOGY

**WOUND HEALING
PROMOTION**

PERINEAL WOUND CLOSURE AFTER APR (Miles procedure)



PILONIDAL DISEASE



OPEN



vs CLOSE

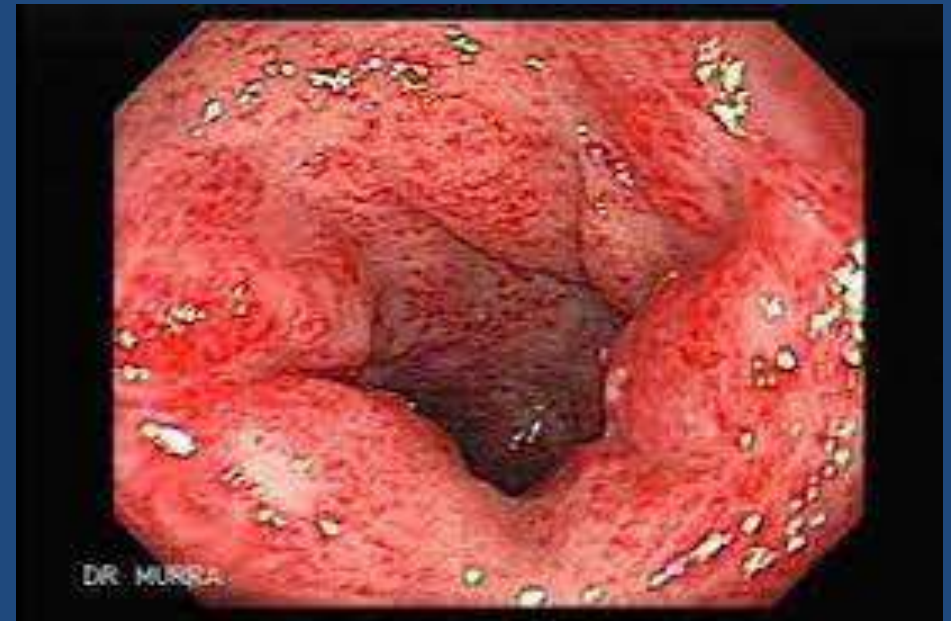


vs MINI INVASIVE

POST RADIOTHERAPY



ANAL STENOSIS



**RADIOATION
PROCTITIS**

COLOPROCTOLOGY

**FECAL
INCONTINENCE**

Intersphincteric anal lipofilling with micro-fragmented fat tissue for the treatment of faecal incontinence: preliminary results of three patients.

Cestaro G, De Rosa M, Massa S, Amato B, Gentile M.

Wideochir Inne Tech Maloinwazyjne. 2015 Jul;10(2):337-41.

...injection, in the intersphincteric anal groove, of lipoaspirate processed by Lipogems...

3 patients...

FU 1 week, 1 month and **6 months**. Improved Wexner incontinence score at 1 month after the procedure. We observed an **increase of resting pressure** (by at least 10 mm Hg) and **thickness of the internal anal sphincter** respectively at ano-rectal manometry and by ultrasound (US) evaluation at the sixth month of follow-up.

Recovery of Function in Anal Incontinence After Micro-Fragmented Fat Graft (Lipogems®) Injection: Two Years Follow Up of the First 5 Cases

A. Giori¹, C. Tremolada², R. Vailati¹, S.E. Navone³, G. Marfia³, A.I. Caplan⁴

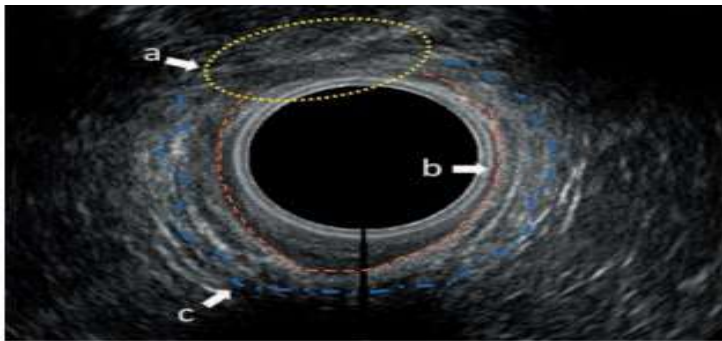


Figure 2. Lipogems injection. Areas of inoculation of Lipogems (endoanal ultrasound image at mid anal canal level). *a*, defect of external and internal anal sphincter; *b*, intersphincteric space; *c*, peripheral zone of the external sphincter.

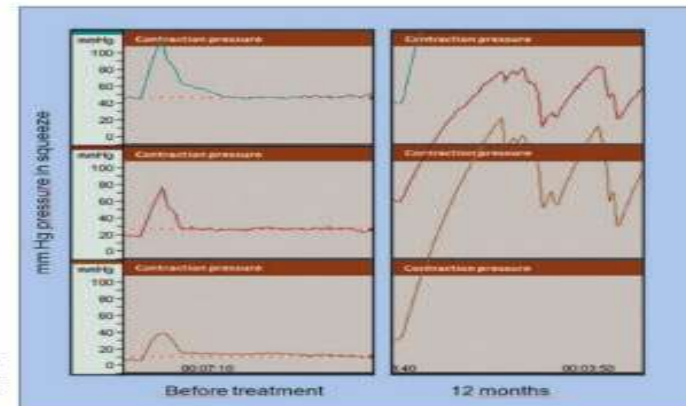


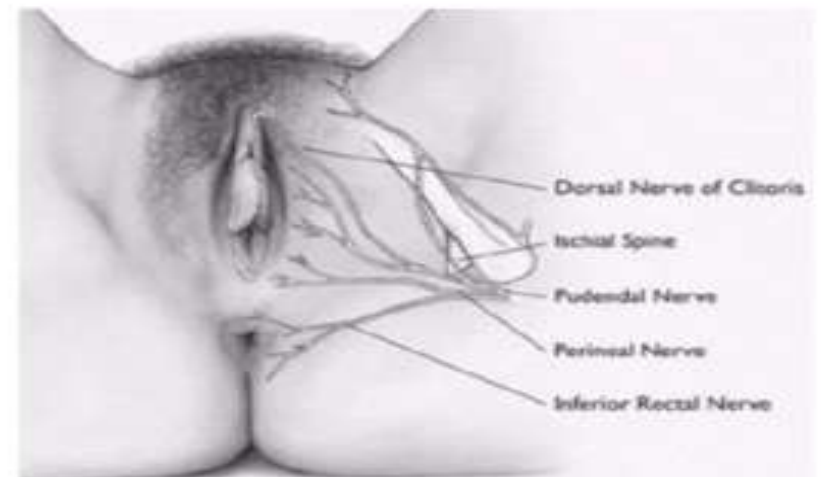
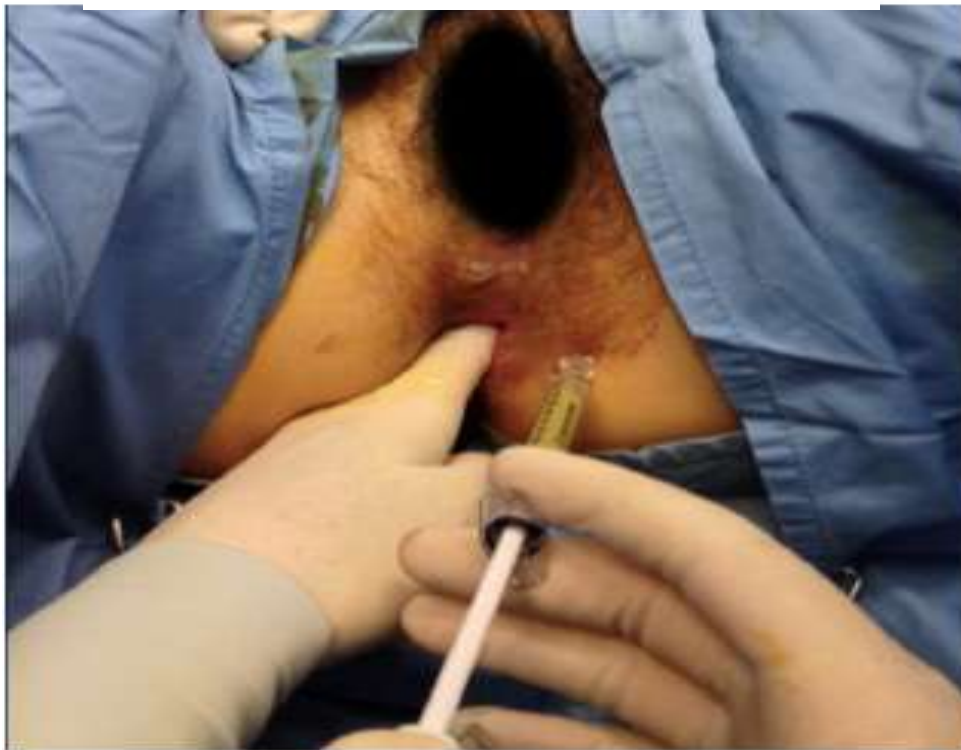
Figure 7. Manometric curves. Patient A: manometric curves in squeeze recorded before and 12 months after Lipogems treatment.

5 patients with fecal *incontinence due to obstetric injury and anorectal-pelvic surgery* were followed up for 24 months after grafting of an average of 90 cc of aspirated and microfragmented fat (Lipogems®) in the **external, internal anal sphincters and around pudendal nerves**

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PUDENDAL NERVE INJECTION

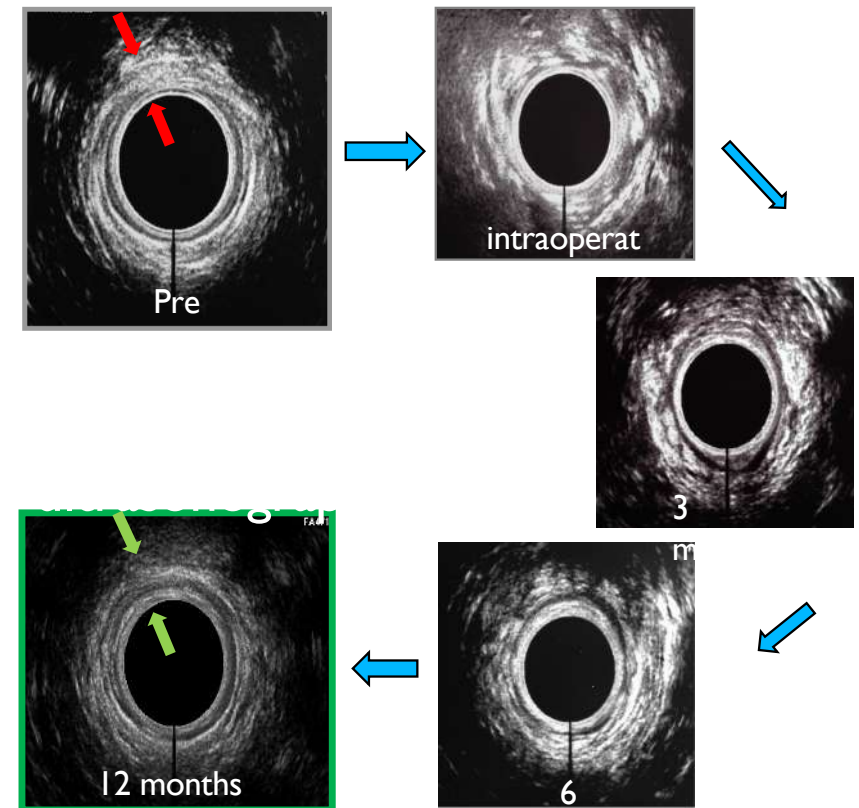


Recovery of Function in Anal Incontinence After Micro-Fragmented Fat Graft (Lipogems®) Injection: Two Years Follow Up of the First 5 Cases

A. Giorì¹, C. Tremolada², R. Vailati¹, S.E. Navone³, G. Marfia³, A.I. Caplan⁴

- All pts observed an improvement both short and long term.
- The **Wexner-Incontinence Score** improved from a preoperative mean of 14.0 to 3.4 at 3 months p.o. and remained stable up to 24 months.
- **Anorectal manometry** has reported over time significant improvements of pressure both at rest and in squeeze.

Ultrasonography showed reabsorption of Lipogems tissue at 6 months and **clear muscle repair at 12 and 24 months.**



“Ti hanno visto alzare la sottana”

GYNECOLOGY

Vulvovaginal dystrophies

- Dermatitis
- Lichen simplex
- Lichen sclerosus
- Lichen planus
- GSM (Genitourinary Syndrome of the menopause)



- **Vulvovaginal dystrophy is a difficult condition to treat**
- **Treatment is limited to topical and symptom management only**
(CO2 laser treatment (Monalisa touch))

- **Patient Reluctance**
- Variety of overlapping symptoms
- Variety of aetiological agents
- Varied & non-specific appearance of lesions
- **No visible lesions despite distressing symptoms**
- Special diagnostic tools not always available
- Changing nomenclature & classification of lesions

Indications

A condition with **significant implications for quality of life**

Most common in post menopausal women

Chronic inflammatory dermatosis

Cause unknown/autoimmune aetiology

Associated with **development of vulval malignancies**

54%

Vaginal Atrophy^{1,2}

31%

Stress Urinary
Incontinence⁴

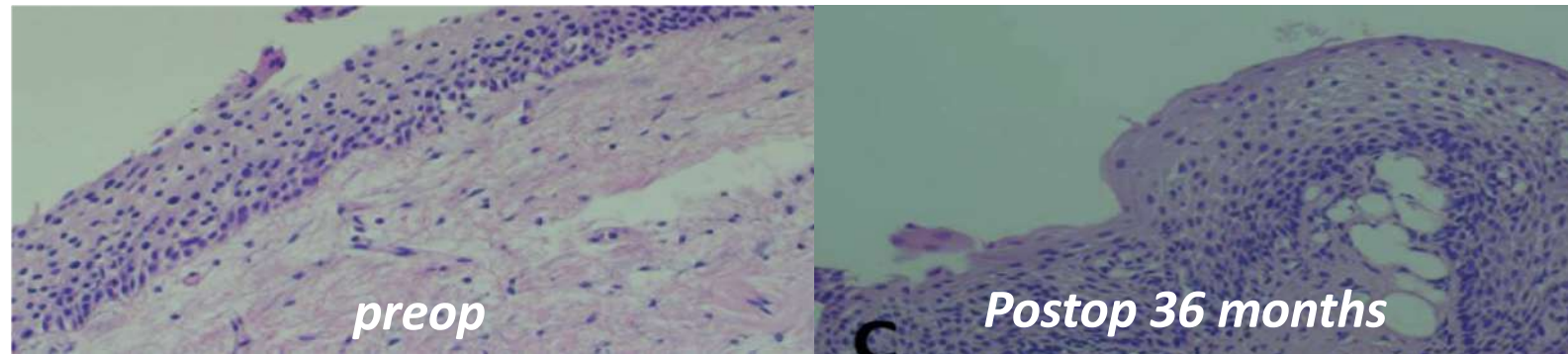
1.7%

Vulvovaginal
Dystrophies³



Menopause: new frontiers in the treatment of urogenital atrophy

European Review for Medical and Pharmacological Sciences 2018; 22: 567-574
G.A. CASAROTTI, P. CHIODERA, C. TREMOLADA - 2018



RESULTS:

1. Lipogems is effective in long term (at least 3 years) **resolution of all the symptoms related to postmenopausal urogenital atrophy** such as **incontinence, dryness, pain**
2. Restoration of **normal PH**
3. **Restoration of premenopausal vaginal hystology**
4. Microfat clusters are present in the chorium 3 years post surgery
5. Increased long term vascularity of chorium



A new treatment of genito-urinary post-menopausal atrophy with autologous micro-fragmented fat tissue: a thirty-six months follow up case series

G. CASAROTTI^{1,2}, C. TREMOLADA^{2,3}

¹Ob/Gyn Specialist, Milan, Italy

²Image Institute, Milan, Italy

³Manchester Metropolitan University, Manchester, UK

35 patients followed for 36 months treated with **single subcutaneous vulvar injection** of autologous micro-fragmented fat tissue (MFAT).

RESULTS:

No adverse effects in all patients. **All symptoms**, and especially pelvic pain and dyspareunia, **improved in almost half of patients within 3 months**. **99% of patients recovered completely from all symptoms after 9-12 months**, reporting **no relapse** of the symptoms up to the 3rd year of follow up.

INCLUSION CRITERIA

- Physiological/early menopause from over 4 years (42-64 y.o.).
- Pharmacological menopause.
- No hormone replacement therapy for more than 4 years.
- Symptomatic.

SYMPTOMS

- Vaginal dryness, burning, itching, stranguria, bladder tenesmus, urine leakage, loss of sensitivity.
- Classified as **MILD – MODERATE - SEVERE**.
- Follow-up at 3-6-9-12-18-24-36 months after the SINGLE TREATMENT.

DYSPAREUNIA

- Pain assessment with **NRS scale** (Numerical Rating Scale: 0 No pain; 1-3 Mild; 4-6 Moderate; 7-8 Strong; 9-10 Very strong).
- Topographical map of the vulvovaginal area (mons pubis, labia majora, urethral orifice, prepuce, clitoris, frenulum, vestibulum, labia minora, hymen, fossa navicularis, and fourchette).

METHODS

Lipogems® (15/20 ml average) was gently injected with a 20G blunt cannula in small amounts into the ***subcutaneous and sub epithelial vaginal space*** in a homogeneous and uniform manner following the *topographic map indicating the painful and sensible sites*.

In those patients suffering also from **stranguria**, with urine leakage when coughing, the **para-urethral areas** were also injected for about 3 cm along the urethra course

Surgical Technique

The Injecting phase of the procedure is tailored specifically for each patient



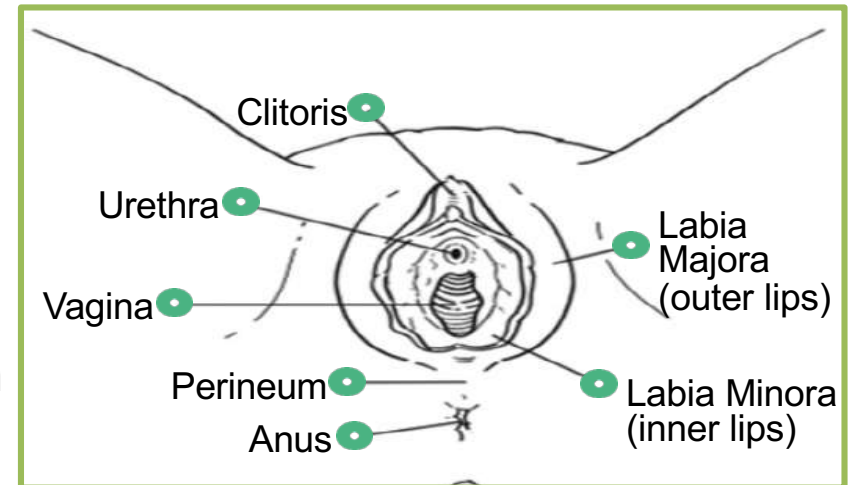
Micro-fragmented adipose tissue was injected using a 20G blunt cannula.



An average of 24ml micro-fragmented adipose tissue was injected per case, range from 8.5mls to 32mls,



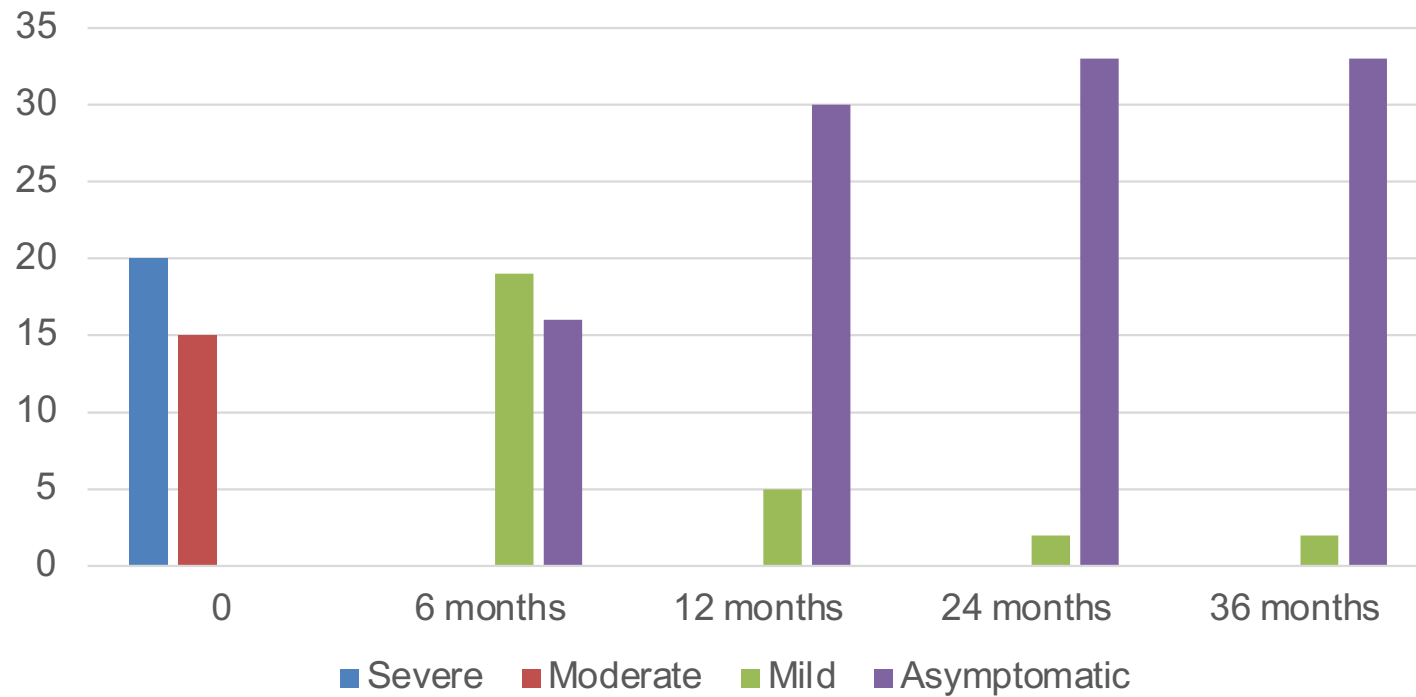
The injections into the genito-urinary took on average 20-30 minutes.



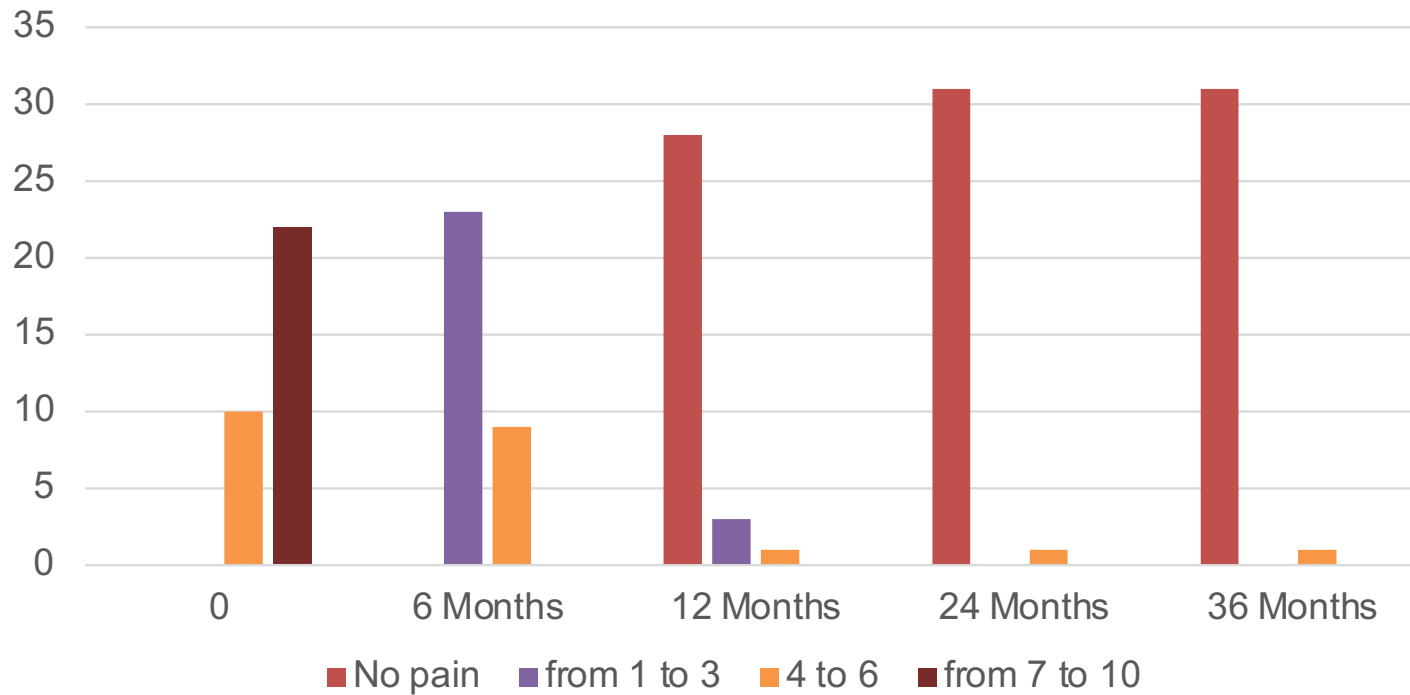
Lichen Simplex: Before and After



DISTRIBUTION OF ALL SYMPTOMS AT DIFFERENT FOLLOW-UP

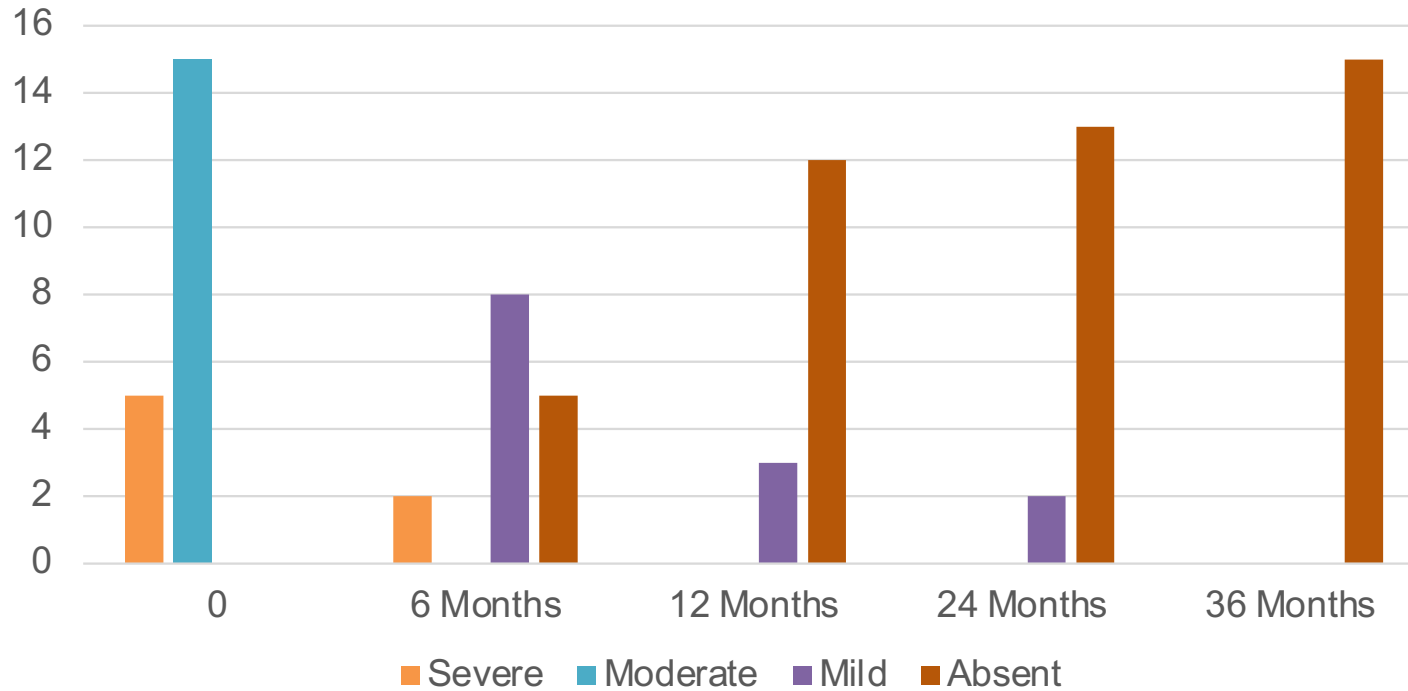


DYSPAREUNIA OVER THE TIME IN 32 PATIENTS

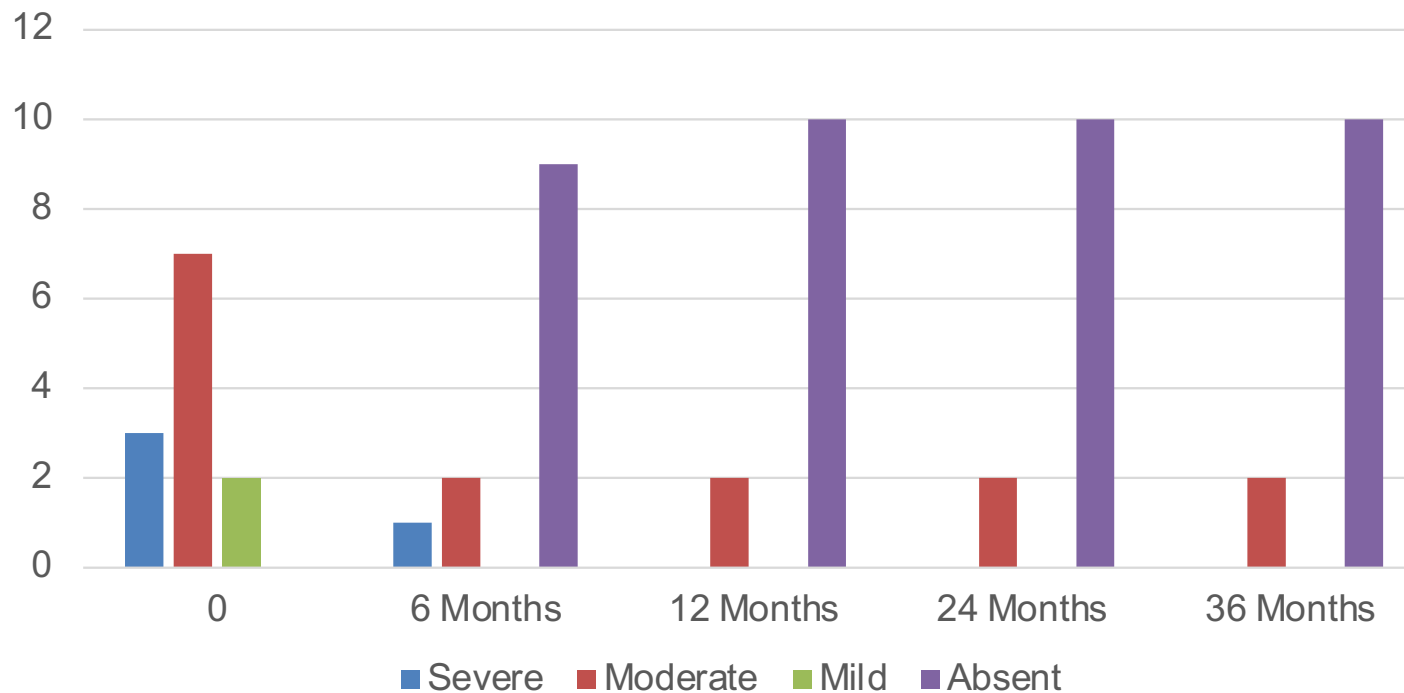


NRS scale

STRANGURIA AND BLADDER TENESMUS OVER THE TIME IN 20 PATIENTS



URINE INCONTINENCE OVER THE TIME IN 12 PATIENTS



**Autologous adipose tissue graft in the vulva in severe vulvar
lichen sclerosus atrophicus: clinical case**

Se Vita D. et al.

Italian Journal of Gynecology & Obstetrics. 2016; 28 (5)

LICHEN SCLEROSUS GENITALIS

- Disease of unknown aetiology, with high symptomatic representation
- 1-5% risk of developing a malignant tumour
- **EGF** (**E**pidermal **G**rowth **F**actor) and its receptor (**EGFr**) reduction

IN VULVA LICHEN SCLEROSUS



Severe Lichen Sclerosus: Before and After



TAAM for Perianal CD

ON THE HORIZON

- ✓ WHAT IS THE IDEAL DOSE?
- ✓ ARE REPEAT INJECTIONS BETTER ?
- ✓ HOW LONG DO THE EFFECTS LAST ?
- ✓ RISKS (infection, tumor) ?
- ✓ RECTOVAGINAL FISTULAS ?
- ✓ PROCTITIS?
- ✓ TISSUE ENGINEERING: COMBINATION WITH OTHER AGENTS AND / OR SURGICAL PROCEDURE (delivery on a plug / seton...)
- ✓ VITRIFICATION OF TAAM / BANKING
- ✓ INTRAARTERIAL DELIVERY (INTERVENTIONAL RADIOLOGY)

TAAM for Perianal CD

ON THE HORIZON

Further clinical trials comparing dose and delivery mechanism will help answer these questions.

- ✓ **EXTEND THE INDICATIONS ?**
- ✓ **CHANGE POSITION IN THE TREATMENT ALGORITHM (TOP – DOWN)?**

STAY TUNED!

Thanks for your attention



silvio.laureti2@unibo.it