

Réinjection de Tissu Graisseux & Dérivés: - Les étapes pour bien débuter.

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FAT

1893

NEUBER *G.A.* 1850 – 1932 **GERMANY**

Fett transplantation, Verl.Dtsch.Ges.Chir. 1893, Long Vern 22:66



CZERNY V.

1842 – 1916 **GERMANY**

Czerny V. Drei plastische Operationen.3. Plastischer Ersatz der Brustdruse durch ein Lipom. Arch f Klin Chir 50:544-550, 1985

LEXER E.

1867 – 1937

GERMANY

Freie Fetttransplantation. Dtsch. Med.Wochenschr. 1910, 36:640





1984 - 1989

ILLOUZ Y.G. 1980 FRANCE

L'avenir de la réutilisation de la graisse après liposucion.
Rev. Chir. Est. 1984, 36 (9)
L'avenir de la réutilisation de la graisse après liposucion.(suite)
Rev. Chir. Est. 1985, 38 (10): 19-22
Utilisation de la graisse aspirée pour combler les defects cutanés.
Rev. Chir. Est. 1985, 40: 13-20
The fat cell graft: A new technique to fill depressions
Plast.Reconstr.Surg. 1986, 78: 122-123

La sculpture chirurgicale par lipoplastie. Perspective d'avenir : la réinjection ou transposition autogène de la graisse aspirée. Paris, Arnette, 1988

Present results of fat injection. Aesth. Plast. Surg. 1988, 12: 175-181

FOURNIER P. 1996 **FRANCE**

Liposculpture: Ma Technique (2ème édition) Paris, Arnette 1996









1994

COLEMAN S.R.

1990 **USA**

- * The technique of periorbital lipoinfiltration Op. Techn. Plast. Reconstr. Surg. 1994, vol 1, n°3
- * Lipoinfiltration of the upper lip white roll. Aesth. Surg. 1994, vol 14, n°4
- * Long-term survival of fat transplants:
 Controlled demonstrations.
 Aesth.Plast.Surg. 1995, vol 19: 421-425
- * Facial recontouring with lipostructure.

 Clinics in Plast. Surg. 1997, vol 24:347-367
- * Training Course, Marseille. 1998





Stromal Vascular Fraction - SVF

1964

RODBELL M. et al.

1969 HOLLENBERG Ch et al.



described Stromal Vascular Fraction (SVF) isolation from adipose tissue using enzymatic digestion

2001

ZUC Patricia A.

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

PATRICIA A. ZUK, Ph.D.,^{1,2} MIN ZHU, M.D.,^{1,2} HIROSHI MIZUNO, M.D.,² JERRY HUANG, B.S.,² J. WILLIAM FUTRELL, M.D.,³ ADAM J. KATZ, M.D.,³ PROSPER BENHAIM, M.D.,² H. PETER LORENZ, M.D.,² and MARC H. HEDRICK, M.D.²





Zuk et al. Tissue Engineering 2001

2013

ZUC Patricia A.

Adipose-Derived Stem cells in tissue Regeneration: A review ISRN Stem Cells, 2013, Article ID 713959



2013

Emulsified Fat

Plastic and Reconstructive Surgery • October 2013

Nanofat Grafting: Basic Research and Clinical Applications

Patrick Tonnard, M.D.
Alexis Verpaele, M.D.
Geert Peeters, M.D.
Moustapha Hamdi,
M.D., Ph.D.
Maria Cornelissen, Ph.D.
Heidi Declercq, Ph.D.

Ghent and Brussels, Belgium

Background: The indications for fat grafting are increasing steadily. In microfat grafting, thin injection cannulas are used. The authors describe their experience of fat injection with even thinner injection needles up to 27 gauge. The fat used for this purpose is processed into "nanofat." Clinical applications are described. Preliminary results of a study, set up to determine the cellular contents of nanofat, are presented.

Methods: Nanofat grafting was performed in 67 cases to correct superficial rhytides, scars, and dark lower eyelids. Three clinical cases are described. In

www.PRSJournal.com

see Video, Supplemental Digital Content 1, http://links.lww.com/PRS/A855



2013

Mixture Fat Grafts and ASCs

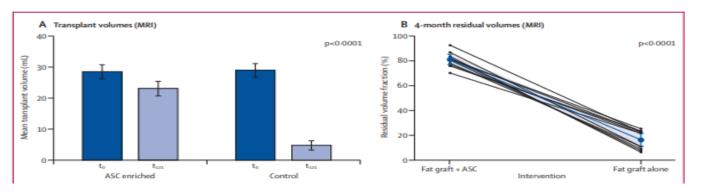


Stig-Frederik Trojahn KOLLE

Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial

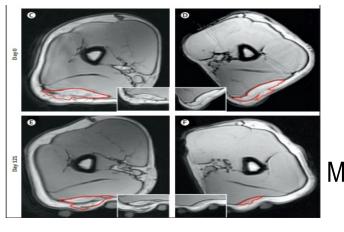


Findings 13 participants were enrolled, three of whom were excluded. Compared with the control grafts, the ASCenriched fat grafts had significantly higher residual volumes: 23.00 (95% CI 20.57-25.43) cm3 versus 4.66 (3.16-6.16) cm³ for the controls, corresponding to 80.9% (76.6-85.2) versus 16.3% (11.1-21.4) of the initial volumes, respectively (p<0.0001). The difference between the groups was 18.34 (95% CI 15.70-20.98) cm3, equivalent to 64.6% (57.1-72.1; p<0.0001). No serious adverse events were noted.



THE LANCET 2013

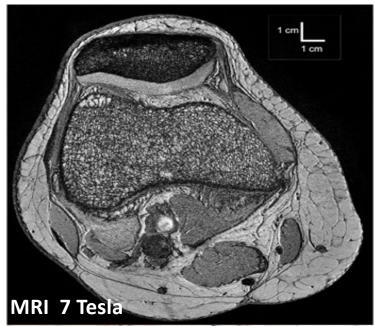
20 millions ASCs / mL de graisse = 650 millions d' ASCs !!! Suivi sur 4 mois...

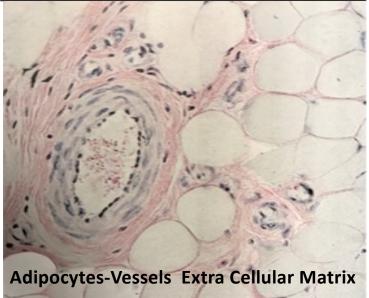


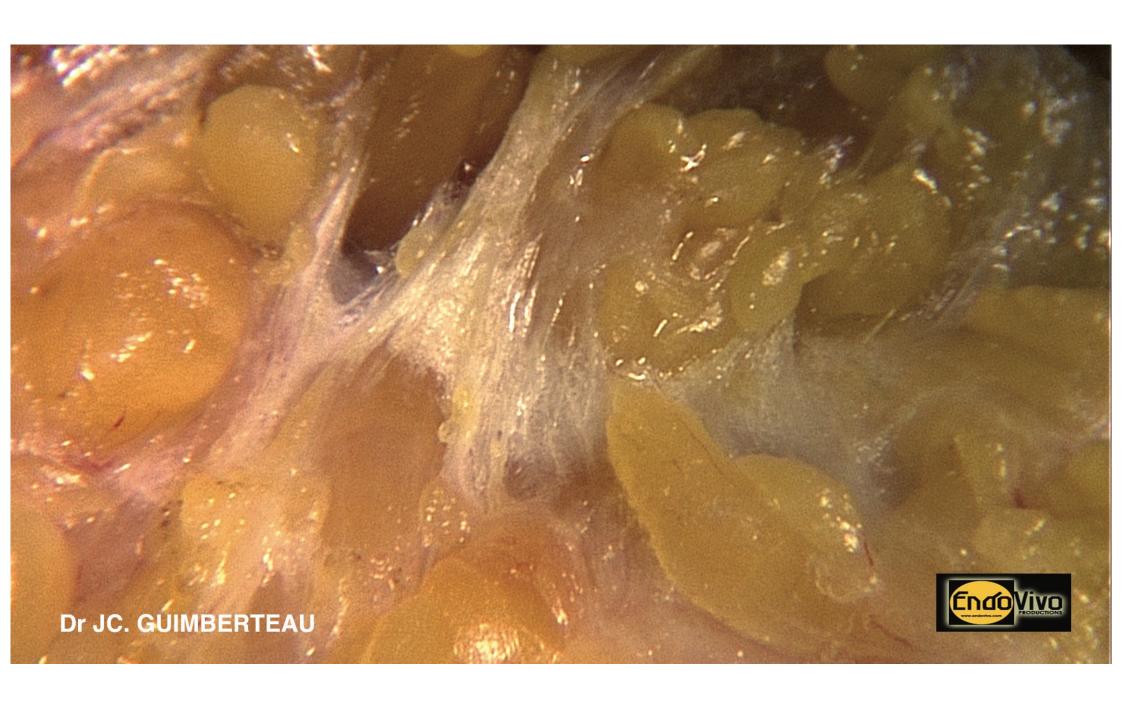
+ ASCs

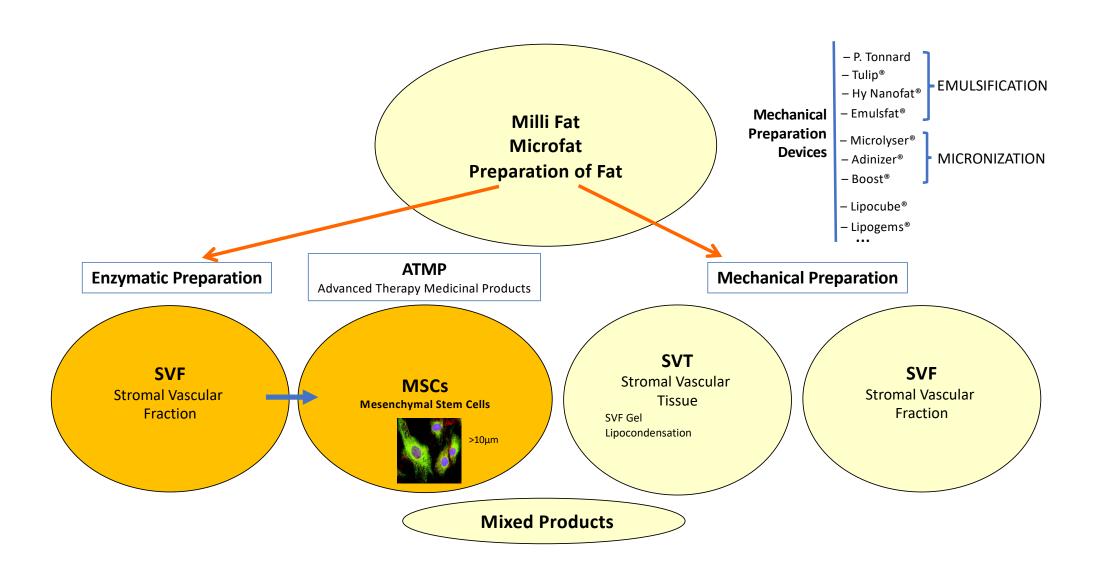
Control

- PRODUCTS
- MEDICAL DEVICES
- TECHNIQUES
 - HARVESTING
 - MANUAL
 - ASSISTED
 - PREPARATION
 - DECANTATION
 - CENTRIFUGATION
 - FILTRATION
 - MECHANICAL PREPARATION
 - INJECTION



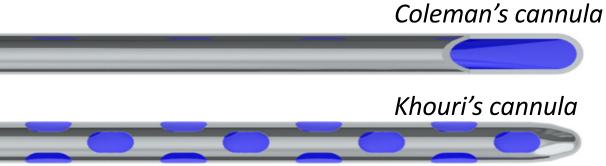






MILLI

Dimensions of Fat Lobules





>/= 2mm

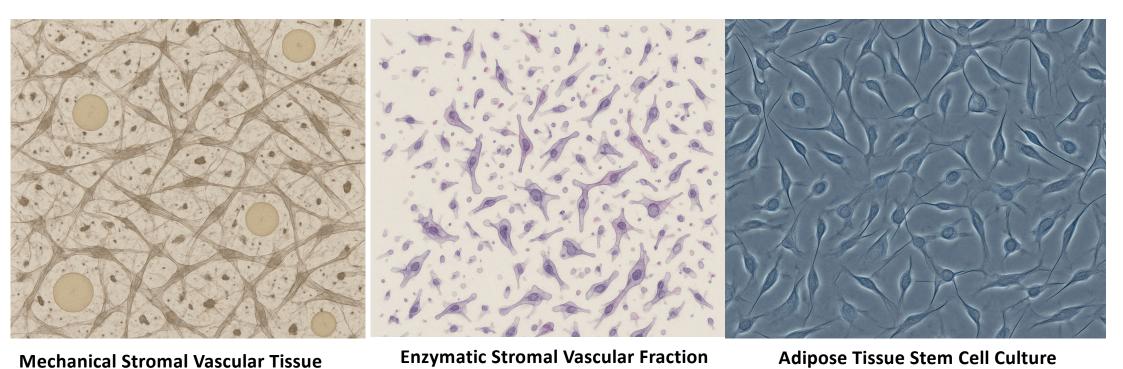
MICRO

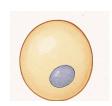
Sorensen's cannula

Magalon's cannula



</= 1mm





















Adipoblast Lymphocyte Macrophage Adipocyte

Fibroblast Endothelial

Endothelial progenitor cell

Pericyte

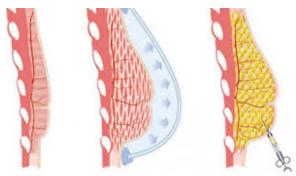
Mesenchymal stem cell

cell

Big volume Fat Harvesting

General Anaesthesia

Breast



Buttock



MicroAire PAL® liposuction system





Alma Lasers





Neosyad

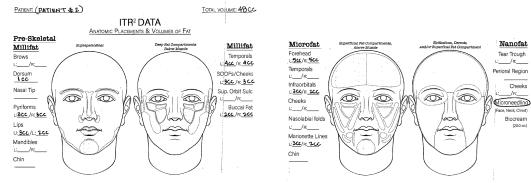


Adimate

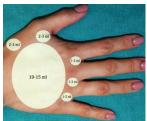
Small volume Fat Harvesting

Local Anaesthesia

• Face



Hand



Genital area



PROTOCOL Local Anaesthesia

Tumescent ANESTHESIA

Klein fluid formula:

• 500 mL of saline solution

25 mL of 1% lidocaine 250mg

• 0,5 mg of epinephrine

6ml of NaH₂CO₃ Sodium bicarbonate Cytori fluid formula: 300ml Fluide 150ml Graisse

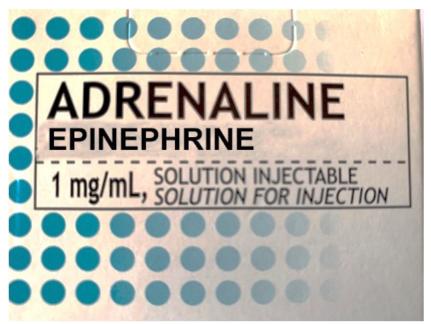
250 mL of LactatedRinger's solution

10 mL of 1% lidocaine100mg

• 0,5 mg of epinephrine



maximum safe dosage 45mg/kg



safe dosage1mg/l

I - MILLI - Sydney COLEMAN



HARVESTING



2,23mm





PURIFICATION



3000 RPM - 3 min -1200G

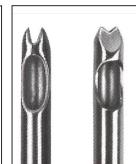


REINJECTION



1mm incision with a #11 surgical blade





17G cannula (O.D.=1,50mm)

Distal opening 2 mm

1 ml LUER LOCK syringue

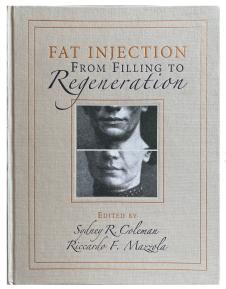


S.R.COLEMAN's placement cannula: 17 G

Cannula creates several tunnels

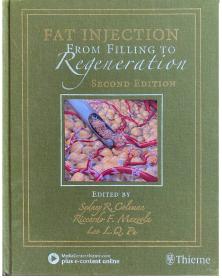


2009



2018

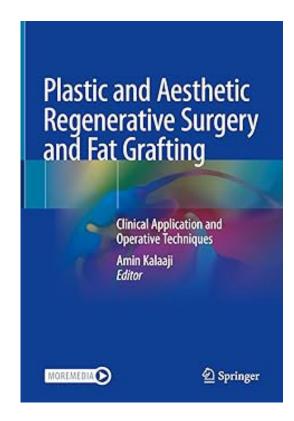
Edited by Sydney R. Coleman



Translated into Chinese

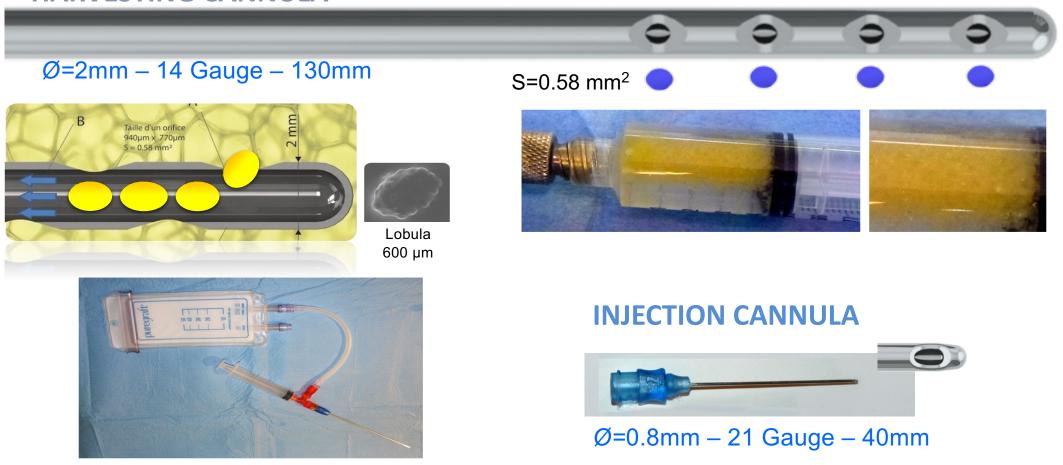


2022



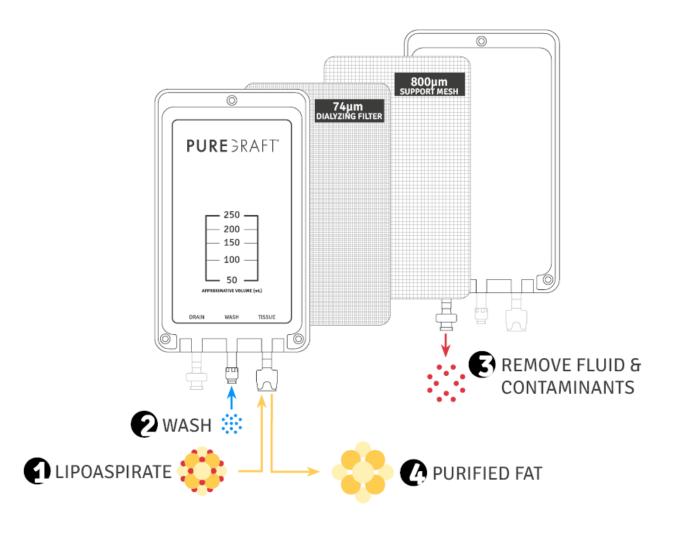
II - MICRO material

HARVESTING CANNULA



FILTRATION BAG CLOSED SYSTEM

FILTRATION BAG











Mesenchymal Stromal Cells (MSC)

Hitomi Eto, M.D. Harunosuke Kato, M.D. Hirotaka Suga, M.D. Noriyuki Aoi, M.D. Kentaro Doi, M.D. Shinichiro Kuno, M.D. Kotaro Yoshimura, M.D.

Tokyo, Japan

The Fate of Adipocytes after Nonvascularized Fat Grafting: Evidence of Early Death and Replacement of Adipocytes

Plastic and Reconstructive Surgery • May 2012

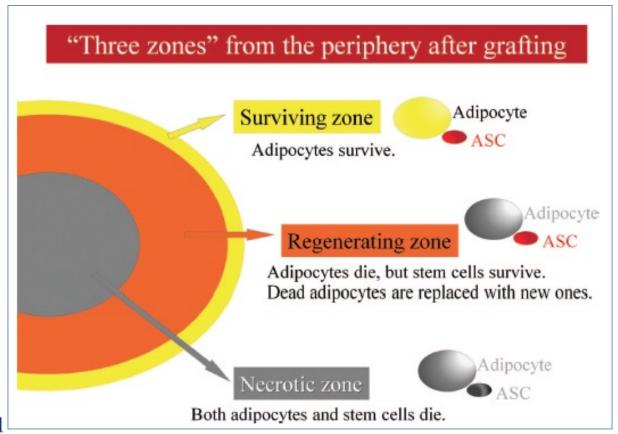


Fig. 6. Conclusive schema for three zones of the grafts. The most superficial zone is the "surviving zone," which is less than 300 μm thick. In the surviving zone, both adipocytes and adipose-derived stromal cells (ASCs) survive. The second zone is the "regenerating zone," the thickness of which varies depending on the microenvironmental conditions such as vascularity of and attachment to the surrounding tissue. In this zone, adipocytes die as early as day 1, but adipose-derived stromal cells survive and provide new adipocytes to replace the dead ones. The most central zone is the "necrotic zone," where both adipocytes and adipose-derived stromal cells die, no regeneration is expected, and the dead space will be absorbed or filled with scar formation.

COSMETIC

Comparison of Three Different Fat Graft Preparation Methods: Gravity Separation, Centrifugation, and Simultaneous Washing with Filtration in a Closed System

Min Zhu, M.D.
Steven R. Cohen, M.D.
Kevin C. Hicok, M.S.
Rob K. Shanahan, B.S.
Brian M. Strem, Ph.D.
Johnson C. Yu, B.S.
Douglas M. Arm, Ph.D.
John K. Fraser, Ph.D.
San Diego, Calif.

Background: Successful long-term volume retention of an autologous fat graft is problematic. The presence of contaminating cells, tumescent fluid, and free lipid in the graft contributes to disparate outcomes. Better preparation methods for the fat graft before transplantation may significantly improve results. Methods: Subcutaneous fat from 22 donors was divided and processed using various graft preparation methods: (1) no manipulation control, (2) gravity separation, (3) Coleman centrifugation, and (4) simultaneous washing with filtration using a commercially available system (Puregraft; Cytori Therapeutics, Inc., San Diego, Calif.). Fat grafts from various preparation methods were examined for free lipid, aqueous liquid, viable tissue, and blood cell content. Adipose tissue viability was determined by measuring glycerol release after agonist induction of lipolysis. Results: All test graft preparation methods exhibited significantly less aqueous fluid and blood cell content compared with the control. Grafts prepared by washing with filtration exhibited significantly reduced blood cell and free lipid content, with significantly greater adipose tissue viability than other methods. Conclusion: Washing with filtration within a closed system produces a fat graft with higher tissue viability and lower presence of contaminants compared with grafts prepared by alternate methods. (Plast. Reconstr. Surg. 131: 873, 2013.)





Effect of Washes and Centrifugation on the Efficacy of Lipofilling With or Without Local Anesthetic

Anne-Claire Girard, PhD*
Sophie Mirbeau, MSc*
Lydie Gence, MSc*
Vincent Hivernaud, MSc*†
Pierre Delarue, MD‡
Olivier Hulard, MD\$
Franck Festy, PhD*
Regis Roche, PhD*

Background: Among the different parameters that influence fat graft survival and lipofilling success, the use of local anesthetic and the way to process the fat before injection have often been pointed out. Likewise, we evaluated different techniques for processing adipose tissue before its injection and analyzed the quality of the grafts.

Methods: Adipose tissue from the same patient was gently harvested from one side of the abdomen after infiltration of a tumescent solution without lidocaine and from the other side of the abdomen using a tumescent solution containing lidocaine 2%. Harvested tissue was prepared with different protocols, from simple decantation to advanced protocols including single or multiple washes and centrifugations. Each type of processed adipose tissue was then injected subcutaneously into immunodeficient mice. Adipose grafts were collected after 1 month and analyzed by histology with a detailed scoring method. Results: After lidocaine use, decantation protocol led to adipose grafts of poor quality with high resorption rate and oil vacuole formation. Larger grafts were obtained after centrifugation, but centrifugation alone resulted in increased fibrosis and necrosis, with or without the use of lidocaine. Finally, multiple washes and centrifugations greatly improved the quality of the lipografts.

Conclusions: Centrifugation alone is not sufficient and must be associated with multiple washes to improve graft quality. This article aims to provide further evidence of lidocaine and washing/centrifugation effects in fat grafting to provide easy tips aimed at ensuring graft efficiency with a long-term clinical outcome. (Plast Reconstr Surg Glob Open 2015;3:e496; doi: 10.1097/GOX.000000000000000465; Published online 27 August 2015.)

III - MECHANICAL PREPARATION OF FAT SVT Stromal Vascular Tissue

EMULSFAT

HY TISSUE -NANOFAT

TULIP







LIPOCUBE



ADINIZER





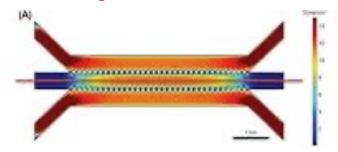






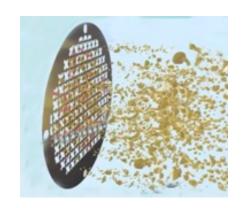
Choose a Mechanical Preparation Kit

• Emulsification: shear force



Micronization





Final size with or without filter:

2400μ 1200μ 600μ 400μ

200μ

 120μ

 100μ



No enzyme - Mechanical production **Inside Operating room** SVF cells - Oil - Adipocytes?

Hôpitaux | CD. Universitaires de Marseille hm

> Ongoing research from Marseille University hospital

2024

BIOLOGICAL QUALITY OF SVF FROM NANOFAT OBTAINED WITH COMMERCIAL DEVICES











BOOST







LIPOCUBE



ADINIZER



91.8 ± 8.5 %

Recovery yield Viable nucleated cells / cc of AT 838 250 ± 636 014





Lower device

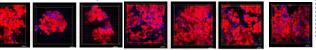
Higher device

83.6 ± 12.4 %

95 550 ± 96 337

Regenerative cells 49.7 ± 20.4 % Leukocytes 28.9 ± 10.5 %

Regenerative Cells 34.3 ± 29.4 % Leukocytes 52.6 ± 11.4 %



ADIPOCYTES IN ALL EMULSIFIED FAT +++

MECHANICAL PREPARATION

- I. LOCATION AND DEPTH OF SAMPLING
- II. INFILTRATION OR NOT?
- III. SIZE AND QUALITY OF THE SYRINGES
- V. CANNULAS

SIZE OF THE HARVESTING CANNULA SIZE AND NUMBERS OF HOLES SHARP MICROPORTS

V. PURIFICATION STEP OF FAT:

DECANTATION
CENTRIFUGATION
FILTRATION

VI. KIT CHOICE:

FAT EMULSIFICATION FAT MICRONIZATION

VII. SIZE OF THE FINAL FILTER

WE NEED VERY PRECISE
PROTOCOL WITH
BIOLOGICAL
CHARACTERIZATION OF THE
FINAL PRODUCT
Each modification changes
the final product

IV - STROMAL VASCULAR FRACTION

ADIPOSE DERIVED REGENERATIVE CELLS - ADRCs

- MANUAL TREATMENT in a clean room or laboratory according to the cGMP
- **AUTOMATIC TREATMENT** using a CE marked device, labeled to replace, repair, reconstruct or increase soft tissue defects.



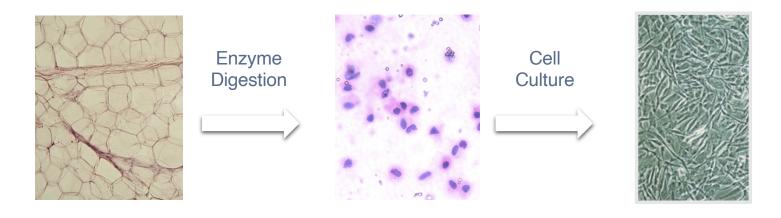


GID TECHNOLOGY™



ACSTM

Adipose-Related Terminology



Aspirated Adipose Tissue

Stromal Vascular Fraction

ADSCs

Cell Type	Frequency	
Adipocytes	~50%	
Blood Vessel Cells	25%	
Blood Cells & Macrophages	25%	
Stem Cells	~1%	

Cell Type	Frequency
Adipocytes	0%
Blood Vessel Cells	~50%
Blood Cells & Macrophages	~50%
Stem Cells	1-2%

Cell Type	Frequency
Adipocytes	0%
Blood Vessel Cells	0%
Blood Cells & Macrophages	0%
Cultured ADSCs	100%

LEGAL ASPECTS RELATING TO THE STEM CELL CRITERIA FOR ATMPS

10.12.2007

EN

Official Journal of the European Union

L 324/121

REGULATION (EC) No 1394/2007 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 13 November 2007

on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004

(Text with EEA relevance)

ATMP: Advanced Therapy Medicinal Products

Cells or Tissues have been subject to **substantial manipulation**, so that biological characteristics, physiological functions or structural properties relevant for the intented indication or regeneration are achieved

OR

Cells or Tissues are **not intented to be used for the same essential function** in the recipient as in the donor **(non homologous use)**

MTI : Médicaments de Thérapie Innovante



European pharmacopoeia 5.6

STERILITY TESTING



⇒AEROBIC and ANAEROBIC inoculation



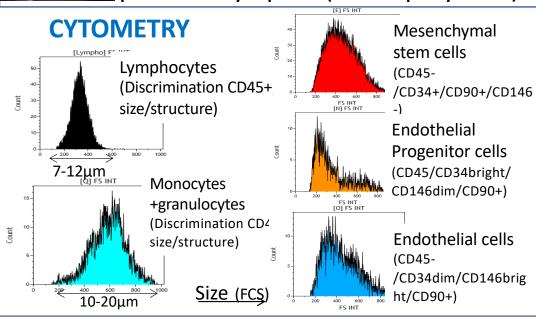
⇒INCUBATION: 10 days

⇒ Recommendations for sampling

Final product volume (ml)	Inoculum volume	
> 10 ml	1%	
1< v <10ml	100 μΙ	

GRAM staining on harvested adipose tissue

prior to delivery to patient (released quality control)



CELL NUMERATION AND VIABILITY

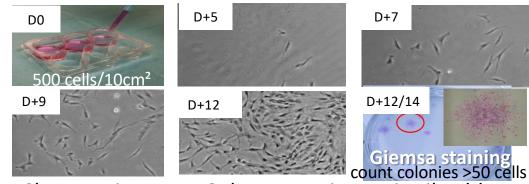
Many methods are available and validated

Choice of automatically method by Nucleocounter NC-100®:

- 15 minutes
- Sample : 100µl
 - Measured in duplicate
 - Propidium iodide
 - 2 steps:
 - Dead cell count
 - Total cell count

⇒SCLERADEC trial : ≥ 80% viability for the batch release

« The current gold standard for enumerating stem cells that are members of the ADSC/MSC family is the CFU-F assay in which the ability of the cells to proliferate is determined. Individual stem cells proliferate to form colonies that are counted. This process takes 10-14 days. » John FRASER



Clonogenic Assay: Colony Forming Unit-Fibroblast
As recommended by the International Society for Cellular Therapy



Enzymatic production ATMPs status Heterogeneous suspension of cells – NO ADIPOCYTES

GMP PRODUCTION & CHARACTERIZATION OF STROMAL VASCULAR FRACTION

RESEARCH

Open Access

Inter-center comparison of good manufacturing practices-compliant stromal vascular fraction and proposal for release acceptance criteria: a review of 364 productions

Pauline François^{1,2}, Giulio Rusconi^{3,4}, Laurent Arnaud⁵, Luca Mariotta³, Laurent Giraudo¹, Greta Minonzio³, Julie Veran¹, Baptiste Bertrand⁶, Chloé Dumoulin¹, Fanny Grimaud¹, Luc Lyonnet⁵, Dominique Casanova⁶, Camille Giverne⁷, Audrey Cras⁸, Guy Magalon⁹, Françoise Dignat-George^{2,5}, Florence Sabatier^{1,2,9}, Jeremy Magalon^{1,2,9*†} and Gianni Soldati^{3†}

2021



294 SVF batches of therapeutic grade produced at **SSCF**



70 SVF batches of therapeutic grade produced at **APHM**

	Viability Ess	Recovery yield Viable nucleated cells / cc of AT	Cellular composition
Swiss Stem Cell N= 294 Foundation	89.3 ± 4.3 %	254 000 ± 120 000	Regenerative cells 70.1 ± 13.1 % Leukocytes 29.8 ± 11.4 %
Hôpitaux Universitaires de Marseille N= 70	84.2 ± 6.0 %	225 000 ± 111 000	ASC 54,8 ± 14.0 % Leukocytes 45.2 ± 16.2 %

V – MIXTURES

CAL: CELL ASSISTED LIPOTRANSFER A POPULAR PROCEDURE...



PRP



ADIPOSE TISSUE





SVT Stromal Vascular Tissue



SVF





Cultured Stem Cells (ASCs) EASY FAST ECONOMICALLY VIABLE



DIFFICULT LONG EXPENSIVE

VI - INDICATIONS

CELLULAR THERAPIES

- Volumizing effect Fat tissue: MILLI MICRO
 - Replacement of missing tissues with autologous fat
- Regenerative effect Fat tissue:
 - -Mechanical preparations STROMAL VASCULAR TISSUE – EMULSIFIED or MICRONIZED FAT
 - Modified emulsified fat SVF Gel
 - Lipocondensation
 - Enzymatic preparation STROMAL VASCULAR FRACTION
- Regenerative effect Platelet Rich Plasma

RULES FOR LIPOFILLING IMPROVEMENT

1 – INFILTRATION

*Careful of lidocaine!

2 - LIPOSUCTION

Small holes cannula = better vascularization Low negative pressure = less than 0.5 atm or 380 mmHg

3 – FAT PREPARATION

Active filtration: removes

- Tumescent fluid, Free lipids, Blood cells ... Washes are really important (even without lidocaine)

4 – FAT INJECTION

Injection of small quantities in different layers

VII - RESULTS

VOLUMIZING

IMMEDIATE EFFECT (2-6 months)

> MILLI

≻MICRO

REJUVENATING

DELAYED EFFECT (12 months)

> MECHANICAL PREPARATIONS :

EMULSIFICATION / MICRONIZATION : "NANOFAT" STROMAL VASCULAR TISSUE

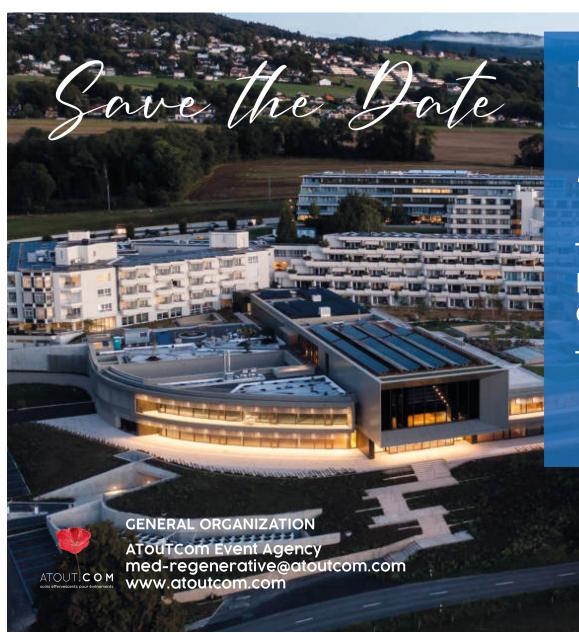
> MECHANICAL PREPARATIONS :

EMULSIFICATION / MICRONIZATION + CENTRIFUGATION : "NON ENZYMATIC SVF"

ENZYMATIC PREPARATIONS: STROMAL VASCULAR FRACTION

CONCLUSION

- Cell Therapy products are manifold, and their preparations require rigorous protocols.
- Regenerative surgery is a major issue.
- Surgeons need to know the products and techniques available and choose indications.
- Advanced Therapy Medicinal Products (ATMP) with substantial manipulations are subject to very strict regulation.
- Evolution:
 - From Enzymatic Stromal Vascular Fraction to Mechanical Modified:
 - Stromal Vascular Tissue: Emulsified Fat Micronized Fat
 - Mechanical Stromal Vascular Fraction
 - Enzymatic SVF or Stem Cells systemic use
 - Stem Cells Expansion System
 - Allogenic Stem Cells
 - Cryopreservation
 - Exosomes



REGENERATIVE MEDICINE & SURGERY

HOW TO SET UP A REGENERATIVE SURGERY STRUCTURE?

FRIDAY, NOVEMBER 14TH, 2025 CLINIQUEGENOLIER-SWITZERLAND

ORGANIZING COMMITTEE

Guy MagalonSophie Menkes

