

CELLULE STAMINALI DA
TESSUTO ADIPOSO NEI
PROCESSI DI RIPARAZIONE
TISSUTALE

Dott.ssa Ilaria Zollino

Medico Chirurgo

Specialista in Chirurgia Maxillo-Facciale

LA STORIA

L'impiego del tessuto adiposo in Chirurgia Plastica trova le sue origini già nel lontano 1893 con il tedesco Neuber



In questo primo periodo, tuttavia, i risultati non erano stati del tutto
soddisfacenti

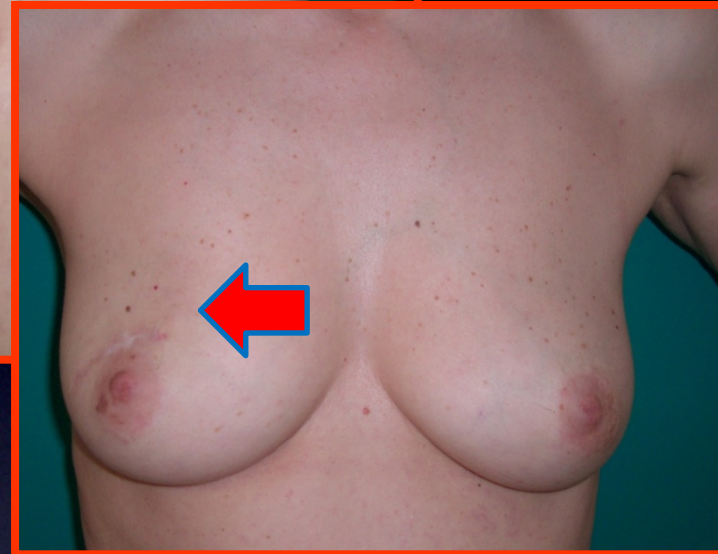


Grazie a **Coleman** (1990) e all'utilizzo delle sue tecniche, il **Lipofilling** ha subito una rivalutazione totale assumendo il termine di **Lipostruttura**

➔ **Il tessuto adiposo non è più solo una fonte calorigena e di deposito, bensì un'inesauribile sorgente di cellule staminali con caratteristiche di totipotenza**

INDICAZIONI

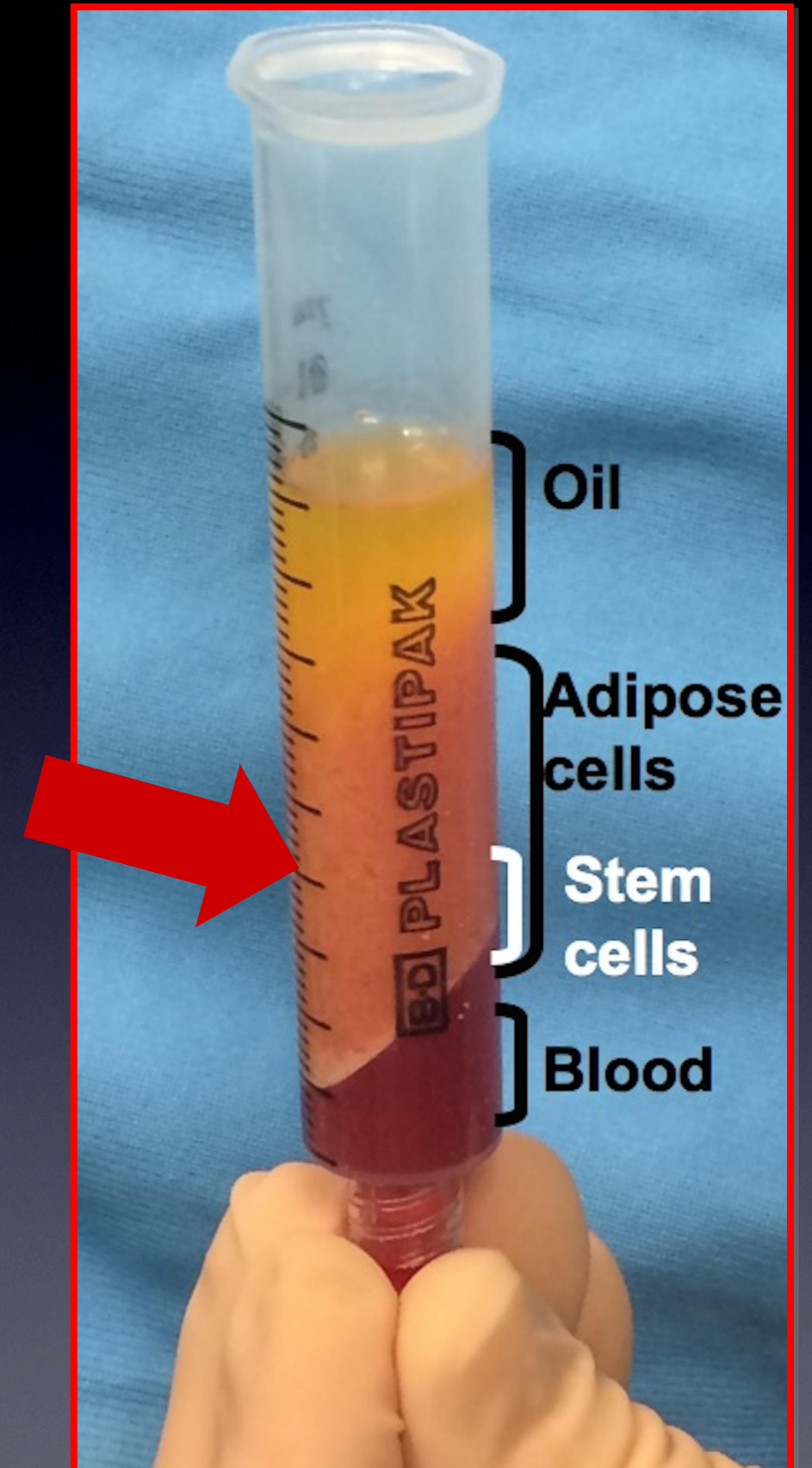
- ✓ *Dismorfismi congeniti*
- ✓ *Perdite di sostanza per cause traumatiche*
- ✓ *Alterazioni volumetriche successive ad interventi chirurgici*
- ✓ *Esiti cicatriziali aberranti*



Si è quindi arrivati a comprendere che all'interno del prelievo non vi è solamente un tessuto adiposo utile a modellare e riempire ma anche un prezioso contingente di *cellule staminali mesenchimali*



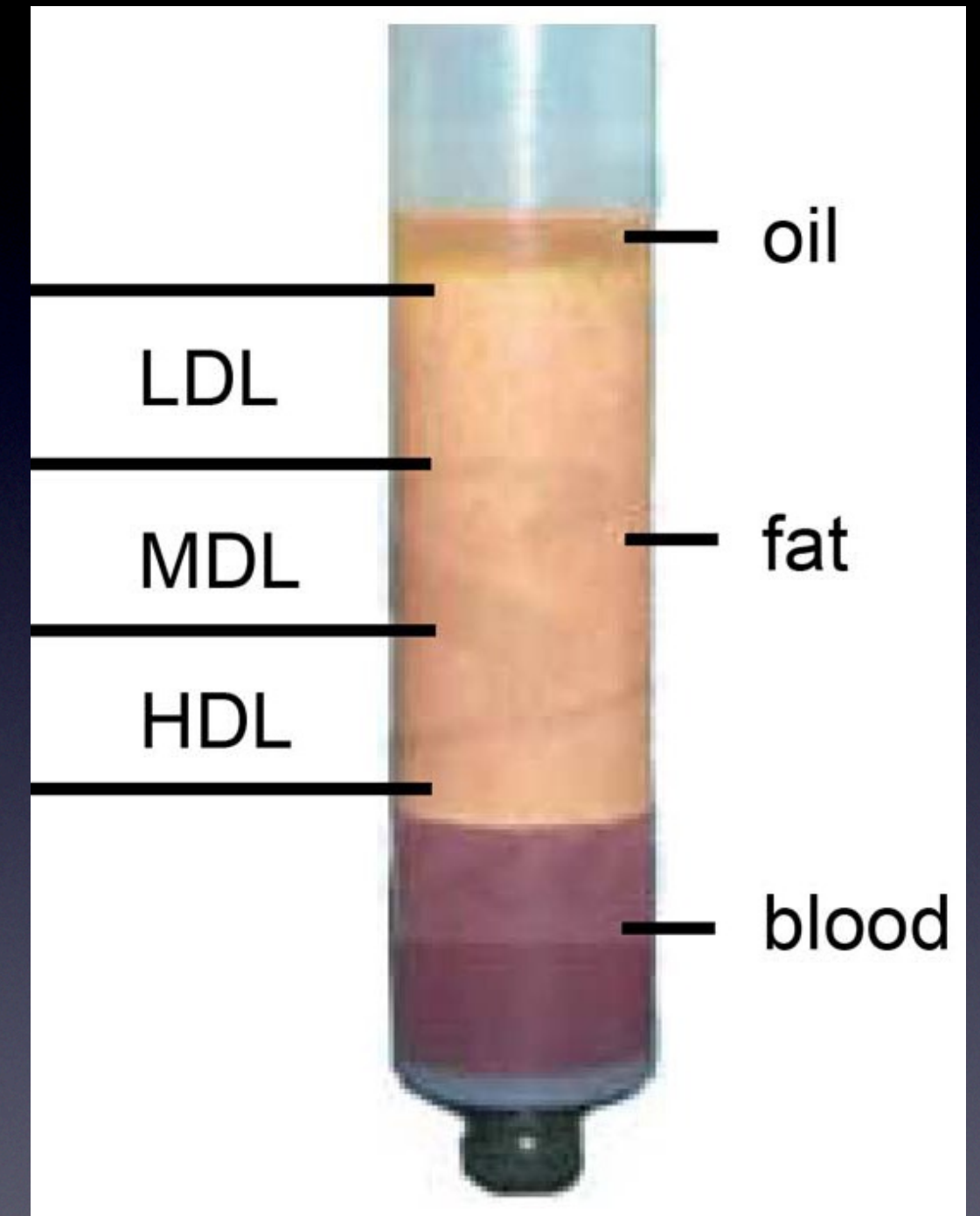
La parte inferiore degli strati adiposi nei centrifugati è particolarmente ricca di cellule staminali



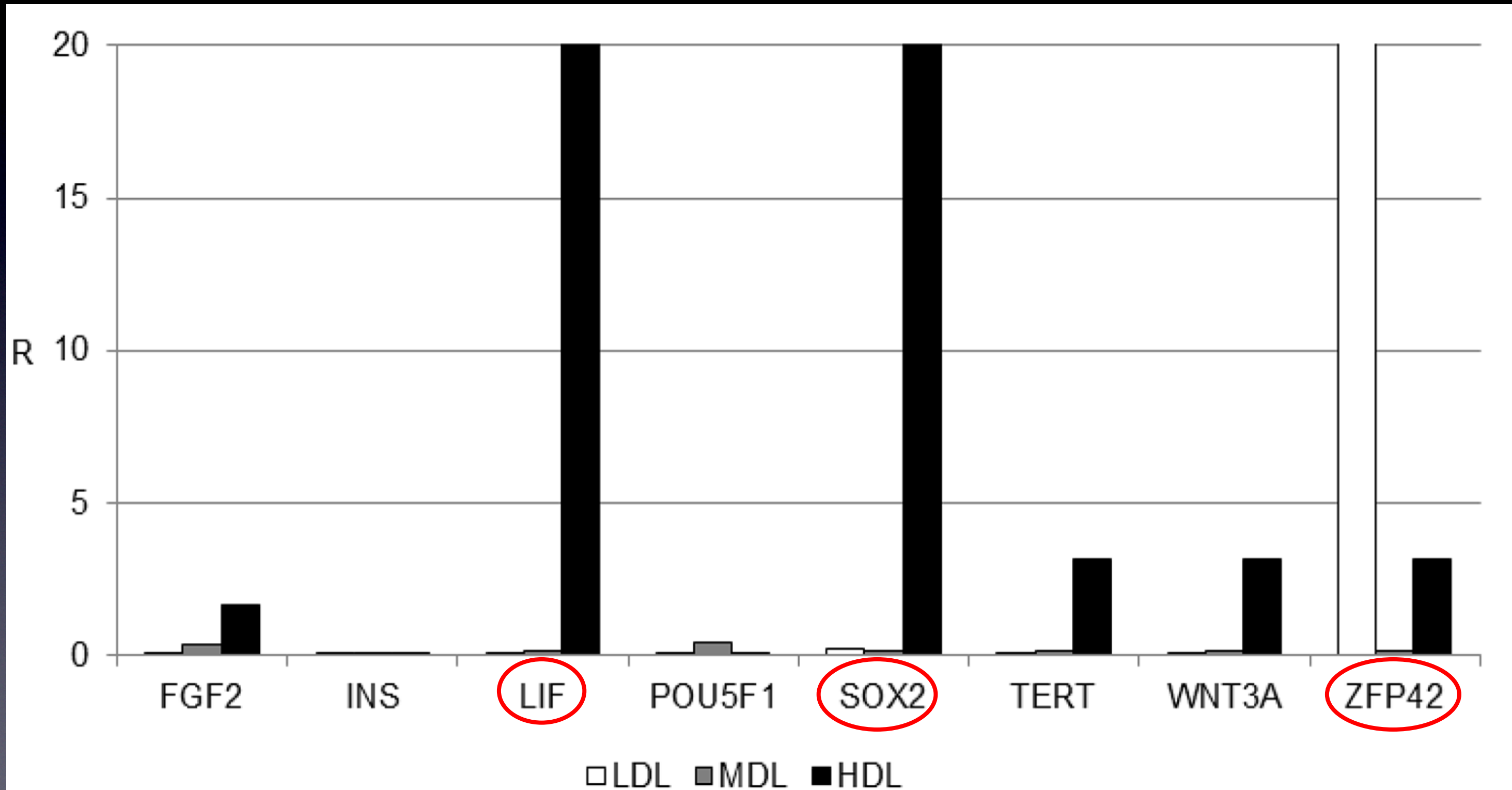
Selective Augmentation of Stem Cell Populations in Structural Fat Grafts for Maxillofacial Surgery

Luigi Clauser^{1*}, Letizia Ferroni^{2,3}, Chiara Gardin², Riccardo Tieghi¹, Manlio Galliè¹, Giovanni Elia¹, Adriano Piattelli³, Paolo Pinton⁴, Erierto Bressan⁵, Barbara Zavan^{2*}

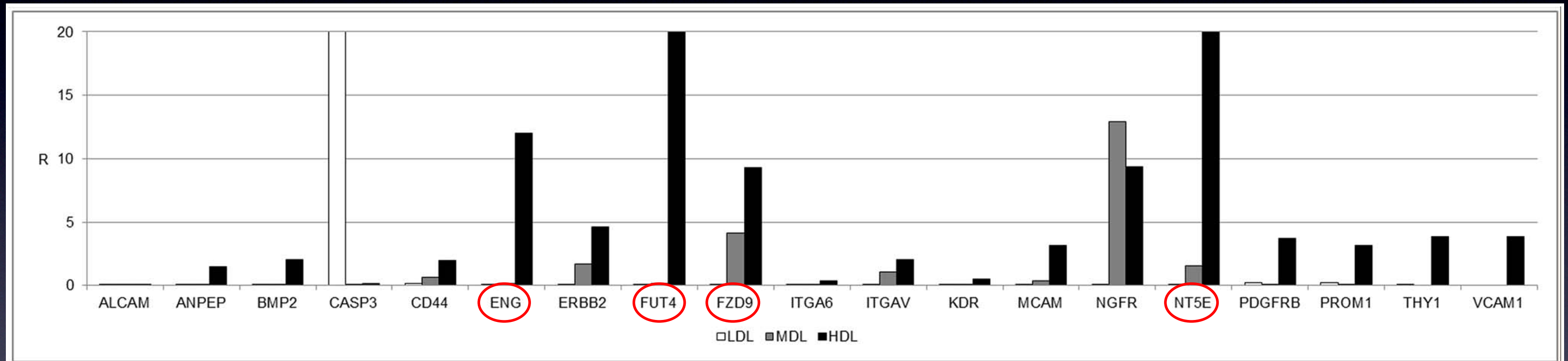
¹Unit of Cranio Maxillo Facial Surgery, Center for Craniofacial Deformities and Orbital Surgery – Reference Center for Rare Disease, Sant’Anna Hospital and University, Cona, Ferrara, Italy, ²Department of Biomedical Sciences, University of Padova, Padova, Italy, ³Department of Medical, Oral, and Biotechnological Sciences, University of Chieti-Pescara, Chieti, Italy, ⁴Department of Morphology, Surgery and Experimental Medicine, Section of Pathology Oncology and Experimental Biology, University of Ferrara, Ferrara, Italy, ⁵Department of Neurosciences, University of Padova, Padova, Italy



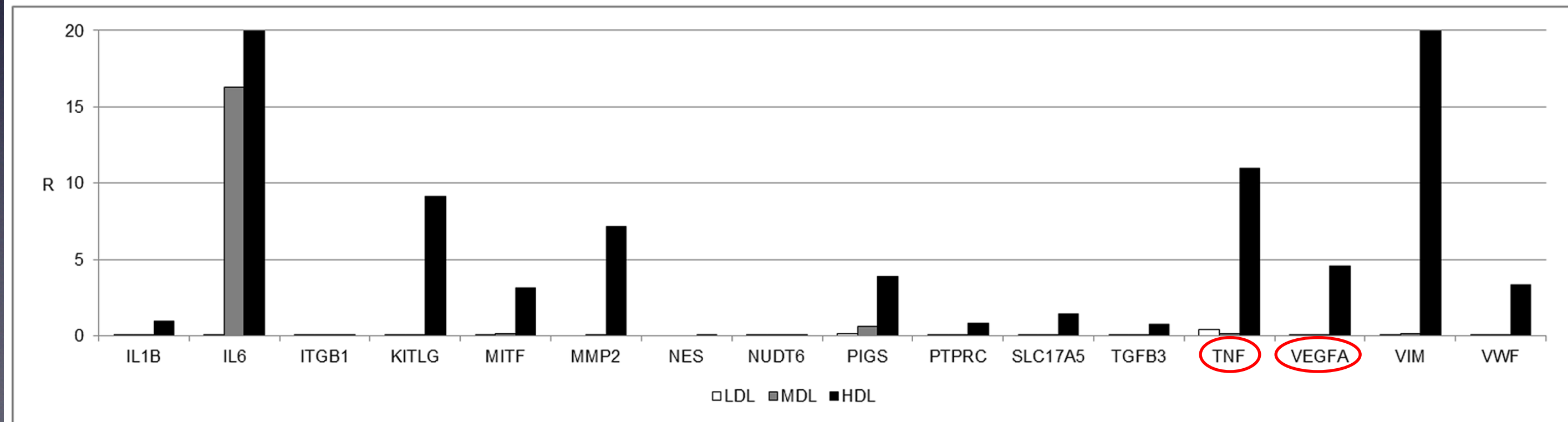
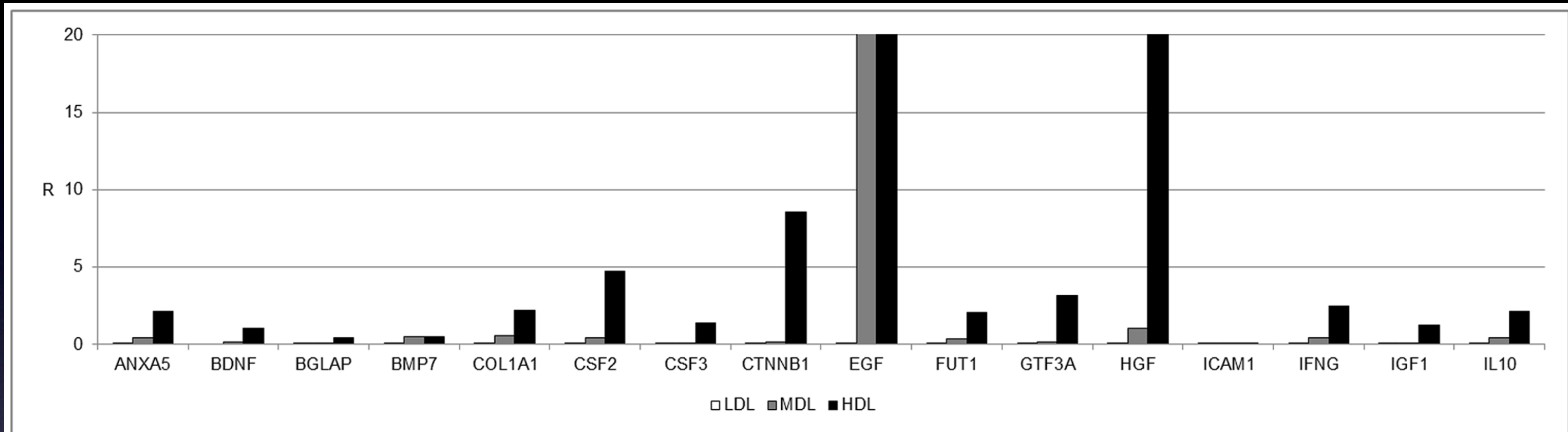
Espressione genica dei markers di staminalità in LDL, MDL, HDL



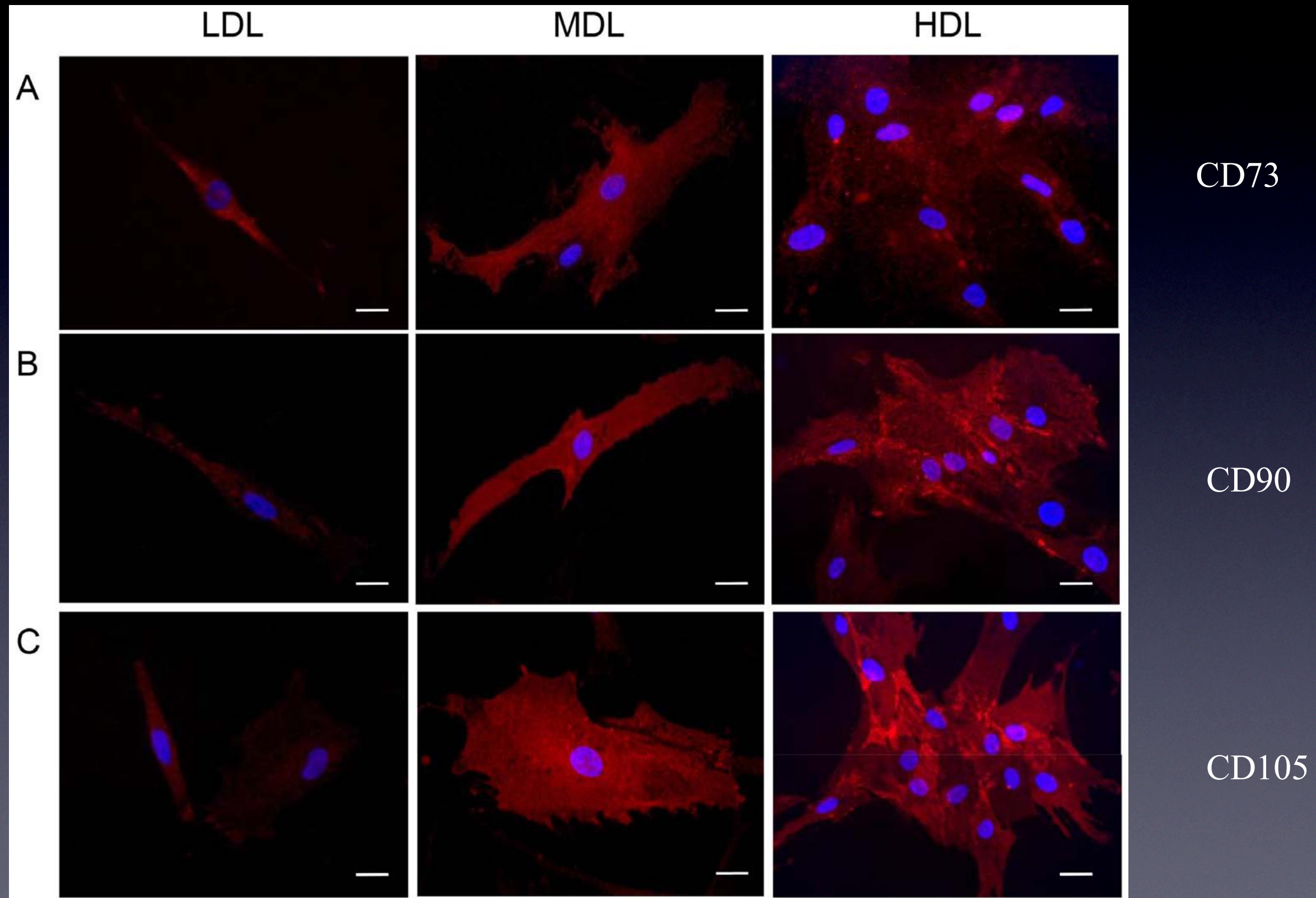
Espressione genica dei markers delle cellule staminali mesenchimali



Espressione genica dei markers delle cellule staminali mesenchimali



Verifica di marcatori di staminalità su cellule delle tre frazioni



CARATTERISTICHE delle CELLULE STAMINALI

DERIVATE da TESSUTO ADIPOSO

(Cellule staminali adulte)

- *ILLIMITATA CAPACITA' DI RIGENERARSI*
- *CAPACITA' DI DIFFERENZIARSI IN LINEE CELLULARI MULTIPLE SOTTO APPROPRIATI STIMOLI*

CELLULE STAMINALI MESENCHIMALI

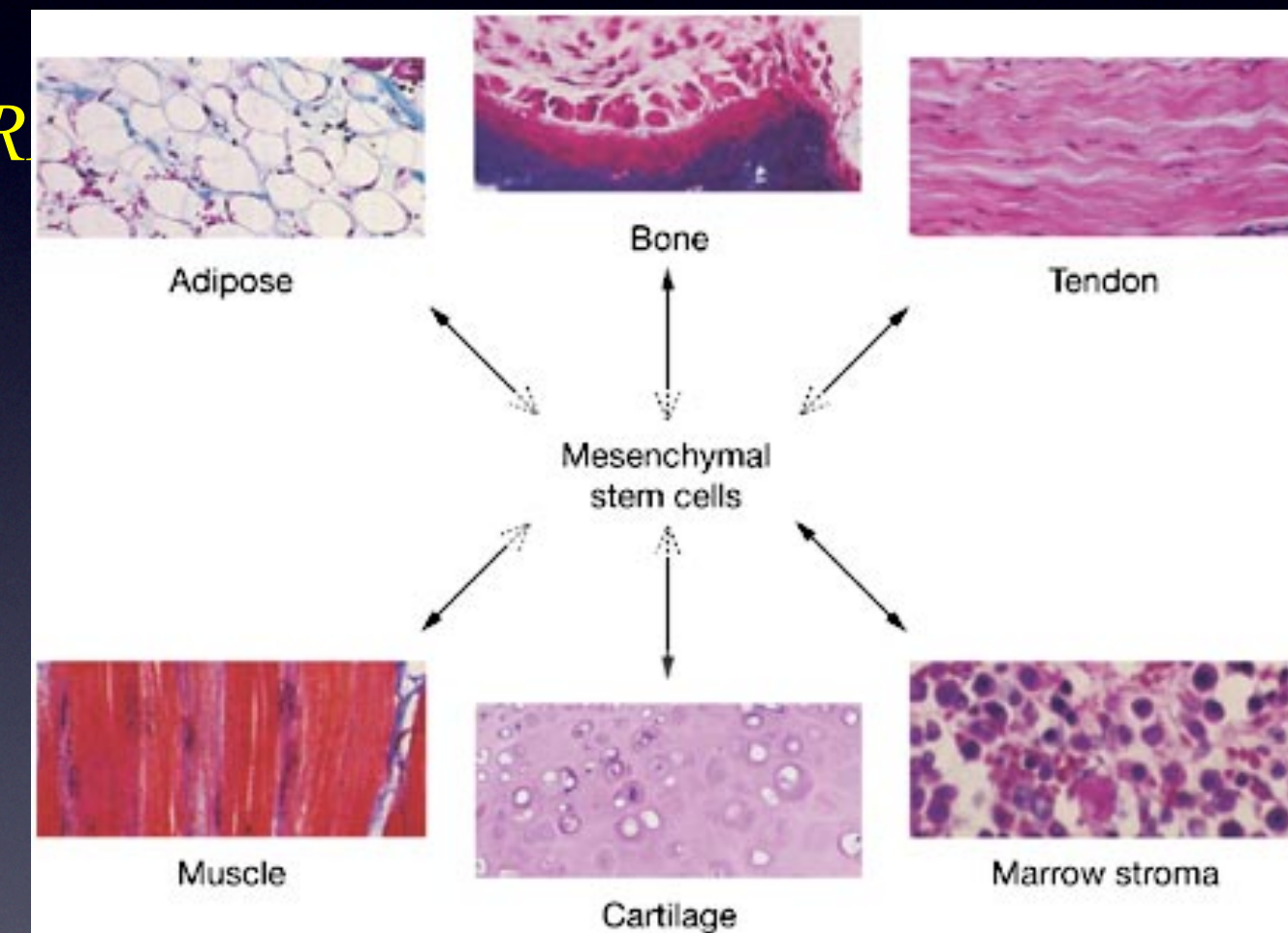
MULTI-POTENTI



OSSO, CARTILAGINE, MUSCOLO e GRASSO

- *CAPACITA' DI SECERNERE UNA GROSSA VARIETA' DI*

FATTORI DI CRESCITA: bFGF, KGF, TGF- β , HGF, VEGF



PERCHÉ LE CELLULE STAMINALI? NELLA MEDICINA RIGENERATIVA

- capacità di IMMUNOMODULAZIONE

(↓ citochine pro-infiammatorie, ↑ citochine anti-infiammatorie)

- meccanismi PARACRINI e sintesi di FATTORI ANGIOGENETICI, di CRESCITA e ANTI-FIBROTICI
- DIFFERENZIAZIONE in fibroblasti e cheratinociti



↑ **RIPARAZIONE** TISSUTALE

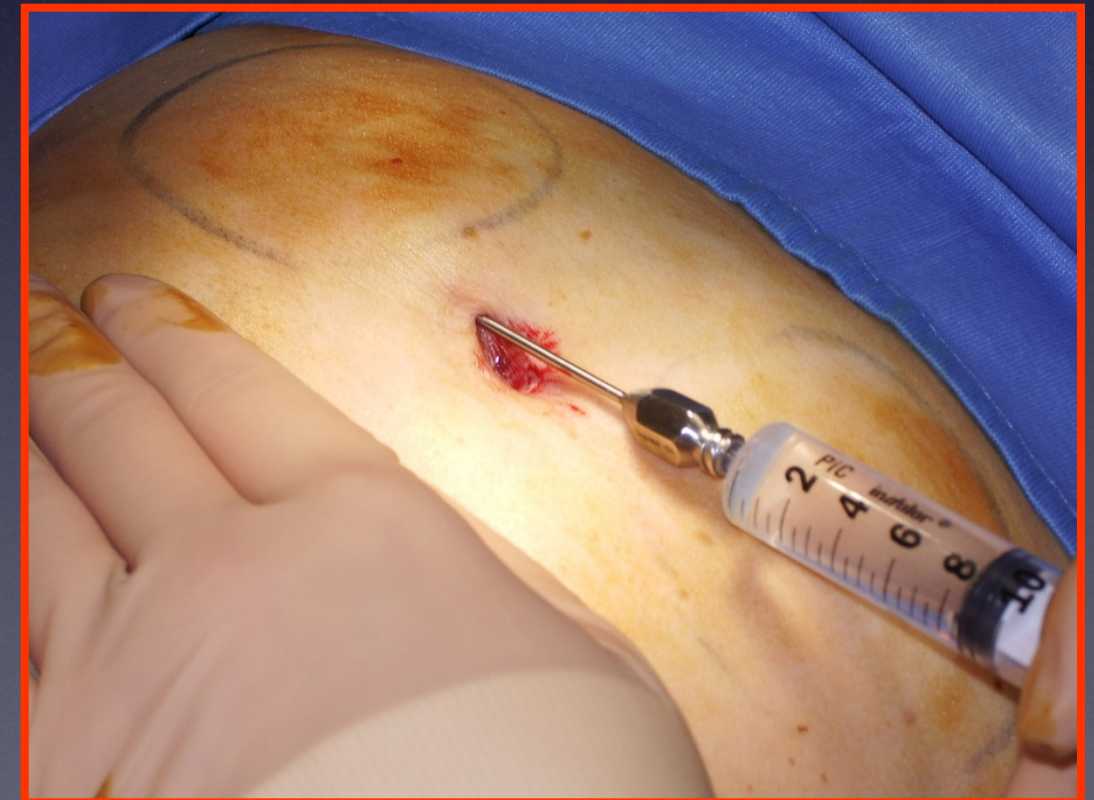
LA TECNICA CHIRURGICA

I TEMPI OPERATORI

1. Anestesia locale
2. Fase del prelievo
3. Fase di trattamento del prelievo
4. Fase di innesto

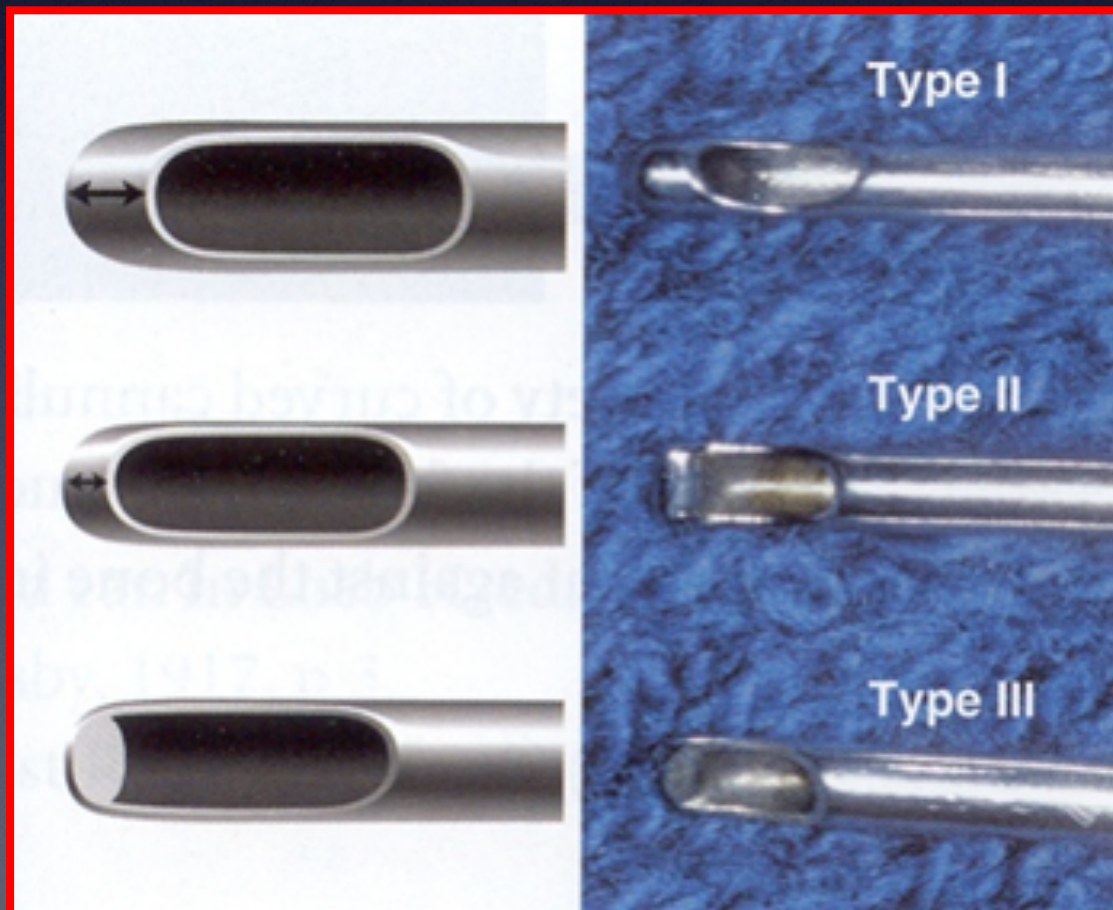
1. Anestesia locale

- Le aree scelte come donatrici (addome e fianchi) vengono disegnate e infiltrate con soluzione di Klein refrigerata (Lidocaina 0,5% in rapporto 1:200.000 ad adrenalina, sodio bicarbonato e soluzione fisiologica)
- Questo permette un'adeguata anestesia dell'area e facilita lo scollamento del tessuto adiposo dai piani contigui



2. Fase del prelievo

- Il tessuto adiposo viene prelevato con un movimento di avanzamento-retrazione dai piani sottocutanei per mezzo di particolari cannule a punta smussa montate su siringhe monouso in cui viene creato l'effetto vacuum



3. Trattamento del prelievo

- Il tessuto prelevato viene sottoposto a centrifugazione (1.300 rpm X 5') per permettere l'eliminazione delle componenti ematica e oleosa

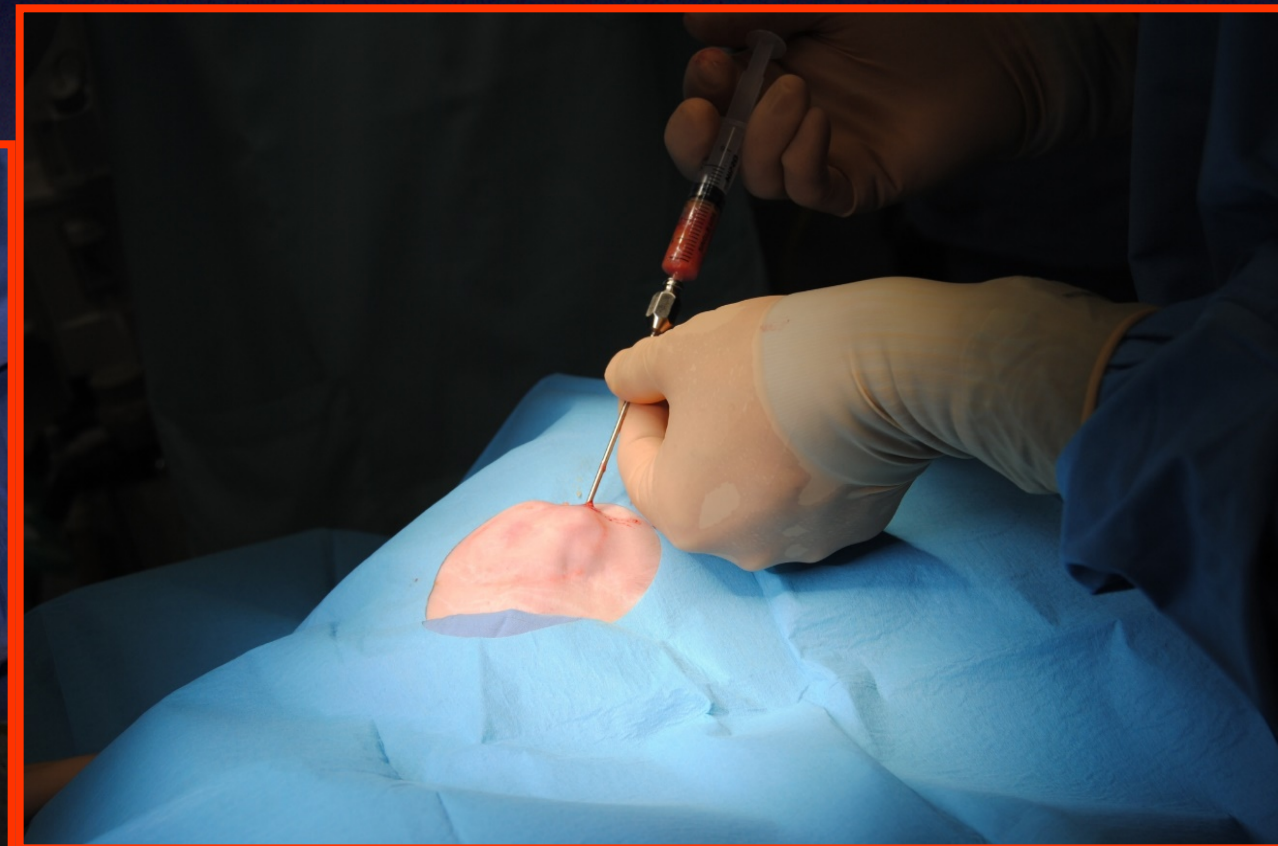


- Viene quindi inserito in siringhe monouso di calibro inferiore



4. Fase di innesto

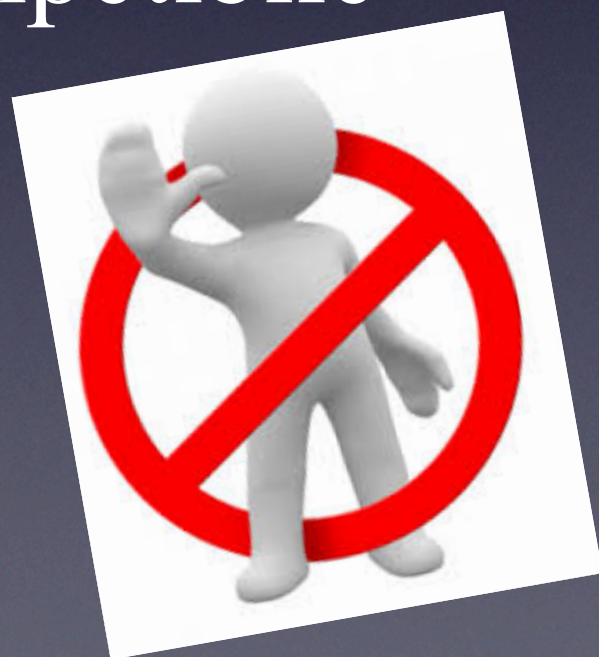
- Il tessuto adiposo è pronto per essere iniettato nelle aree in cui vi sia necessità di integrazione volumetrica



LA TECNICA CHIRURGICA

CARATTERISTICHE

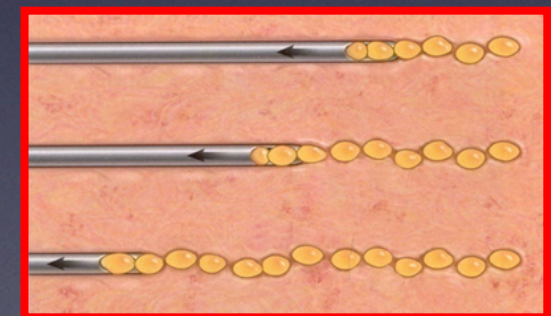
- Tecnica a ridotta traumaticità
- Semplice
- Sicura
- Ripetibile



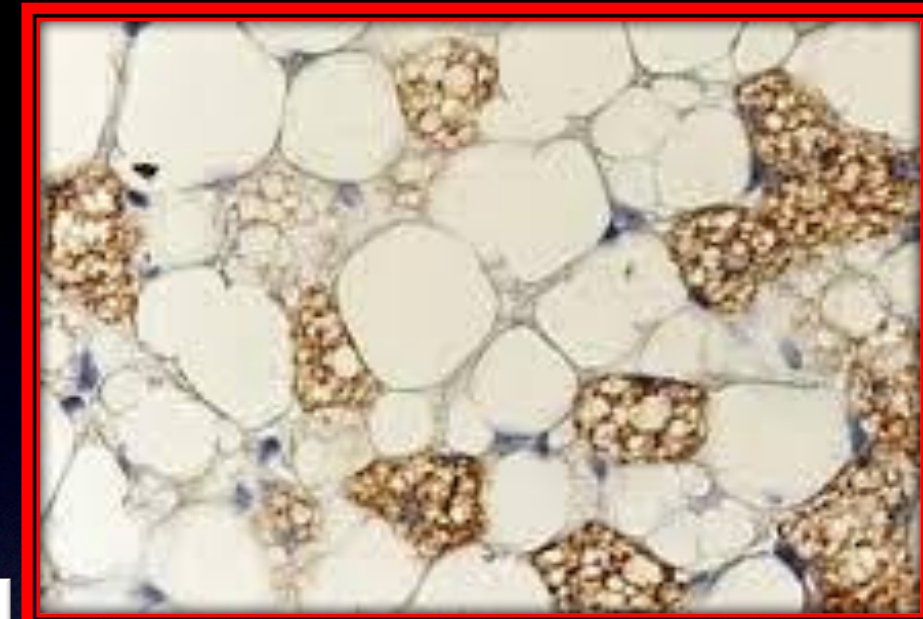
...la curva di apprendimento del chirurgo deve essere molto articolata per evitare di incorrere in possibili errori

I PUNTI CHIAVE

- Seguire e rispettare tutte le fasi del protocollo
- Impiegare un'alta pressione negativa durante la fase di aspirazione del grasso: un'aspirazione meccanica provocherebbe un deterioramento e un danno delle cellule!
- Mantenere nelle fasi di prelievo e di innesto la cannula in posizione orizzontale per evitare danni a nervi e vasi
- Utilizzando una cannula smussa e la centrifugazione non si ha il deterioramento degli adipociti ma una sopravvivenza >90%
 - Iniettare gli adipociti mentre la cannula viene retratta
 - Innestare il grasso in piccole aliquote (0,1 ml) ad ogni passaggio
- L'innesto deve creare nel tessuto ricevente una sorta di griglia tridimensionale



Dalla **CHIRURGIA RICOSTRUTTIVA** ed **ESTETICA** alla **CHIRURGIA RIGENERATIVA**



Phlebology
The journal of venous disease

AUTOLOGOUS ADIPOSE DERIVED STEM CELLS: BASIC SCIENCE, TECHNIQUE, AND RATIONALE FOR APPLICATION IN ULCER AND WOUND HEALING

Journal:	Phlebology
Manuscript ID:	Draft
Manuscript Type:	Review Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Zollino, Iliaria; University of Ferrara, Department of Morphology, Surgery and Experimental Medicine, Section of Translational of Medicine and Surgery Zuolo, Michele; Sant'Anna University Hospital, Unit of Translational Surgery and Vascular Diseases Center GIANESINI, SERGIO; University of Ferrara, Department of Morphology, Surgery and Experimental Medicine, Section of Translational of Medicine and Surgery, Sant'Anna University Hospital, Unit of Translational Surgery and Vascular Diseases Center Pedriali, Massimo; Sant'Anna University Hospital, Department of Experimental and Diagnostic Medicine Sibilla, Maria Grazia; Sant'Anna University Hospital, Unit of Translational Surgery and Vascular Diseases Center Tessari, Mirko; University of Ferrara, Department of Morphology, Surgery and Experimental Medicine, Section of Translational of Medicine and Surgery, Sant'Anna University Hospital, Unit of Translational Surgery and Vascular Diseases Center Carinci, Francesco; University of Ferrara, Department of Morphology, Surgery and Experimental Medicine, Section of Translational of Medicine and Surgery occhionorelli, savino; University of Ferrara, Department of Morphology, Surgery and Experimental Medicine, Section of Translational of Medicine and Surgery, Sant'Anna University Hospital, Unit of Translational Surgery and Vascular Diseases Center Zamboni, Paolo; University of Ferrara, Department of Morphology, Surgery and Experimental Medicine, Section of Translational of Medicine and Surgery, Sant'Anna University Hospital, Unit of Translational Surgery and Vascular Diseases Center
Revised Keywords:	Chronic venous disease, Chronic venous insufficiency, Cosmetic results, Wound care, Vascular surgery

ISSN 0268-3555 Volume 30 Number 10 2015

Phlebology

The journal of venous disease

- Paget-Schroetter syndrome
- Mechano-chemical endovenous using Clariven®
- Maggots as a wound debridement agent
- EVLA in patients with wide diameter vein
- Transvaginal duplex ultrasonography
- Plantar vein thrombosis
- Duplex investigations in children
- RFA of the great saphenous vein
- Arteries masquerading as varicose veins
- Neoplastic zebras of venous thrombosis
- Summaries

The ROYAL SOCIETY of MEDICINE Journals

5.089 *pazienti sottoposti a procedure di chirurgia plastica e maxillo-facciale*

COMPLICANZE: 169 casi (3,3% del totale)

141 (2,77%)  **COMPLICANZE MINORI**

- *nodularità e / o indurimento: 93 (1,83%)*
- *disestesia: 14 (0,26%)*
- *ematoma: 12 (0,23%)*
- *infezione superficiale: 11 (0,21%)*
- *dolore: 7 (0,13%)*
- *scarso risultato estetico: 3 (0,06%)*
- *secrezione seno anomala: 1 (0,02%)*

28 (0,55%)  **COMPLICANZE MAGGIORI (16,5%)**

- *infezione profonda: 22 (0,43%)*
- *sepsi: 3 (0,06%)*
- *ematoma addominale che ha richiesto drenaggio chirurgico percutaneo: 2 (0,04%)*
- *pneumotorace: 1 (0,02%)*

9.245 donatori di midollo

COMPLICANZE: 345 casi (3,7% del totale)

125 (1,35%)



COMPLICANZE MAGGIORI (36,2%)

116 legate al sito di prelievo

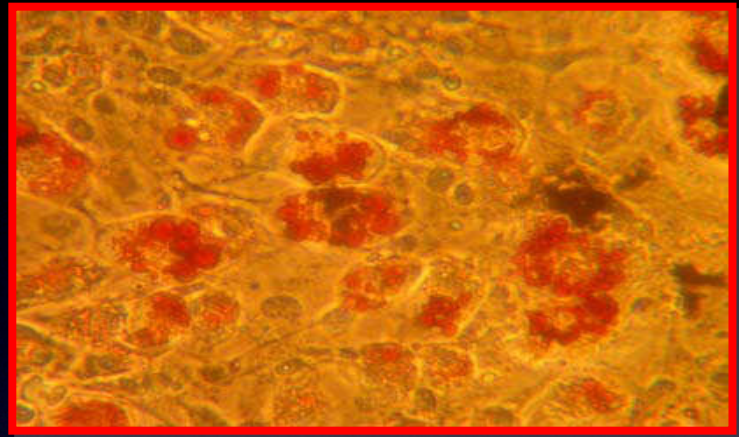
- danno meccanico a tessuti, ossa, nervi: 69 (0,7%)
- anestesia: 45 (0,5%)
- infezione: 1 (0,01%)
- pneumotorace: 1 (0,02%)



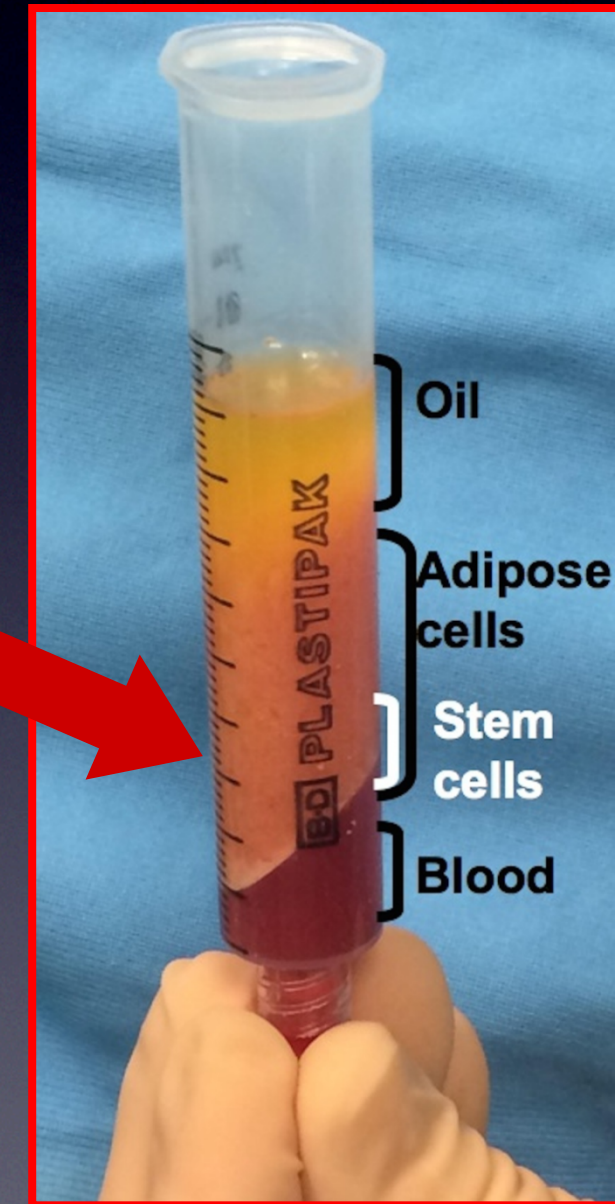
67 pazienti (0,7% dei 9.245): tempi di recupero lunghi (da 1 a 10 anni) relativi al danno meccanico ai tessuti da aspirazione da ago

49 pazienti (0,5% dei 9.245): reazioni acute legate all'anestesia (mal di testa post-spinale, aritmia cardiaca ed edema polmonare)

Vantaggi dell'uso del tessuto adiposo come sito di raccolta prelievo



- Accesso chirurgico semplice
- Alto rapporto numero cellule staminali/ml del tessuto: 4,737 - 1,550,000 (midollo osseo da 1-30 a 317,400)
- Bassa morbidity del sito di prelievo



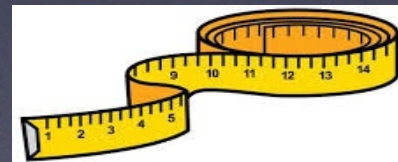
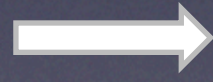
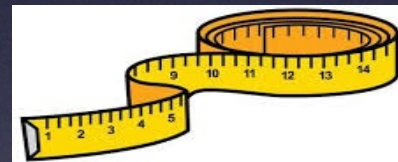
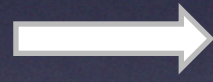
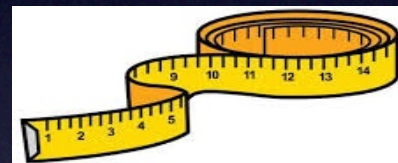
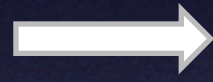
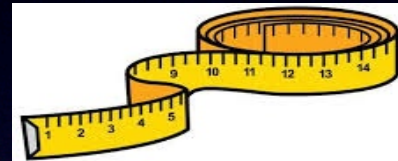
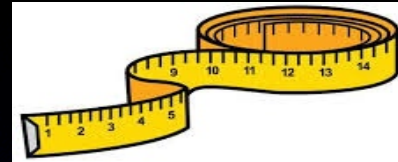
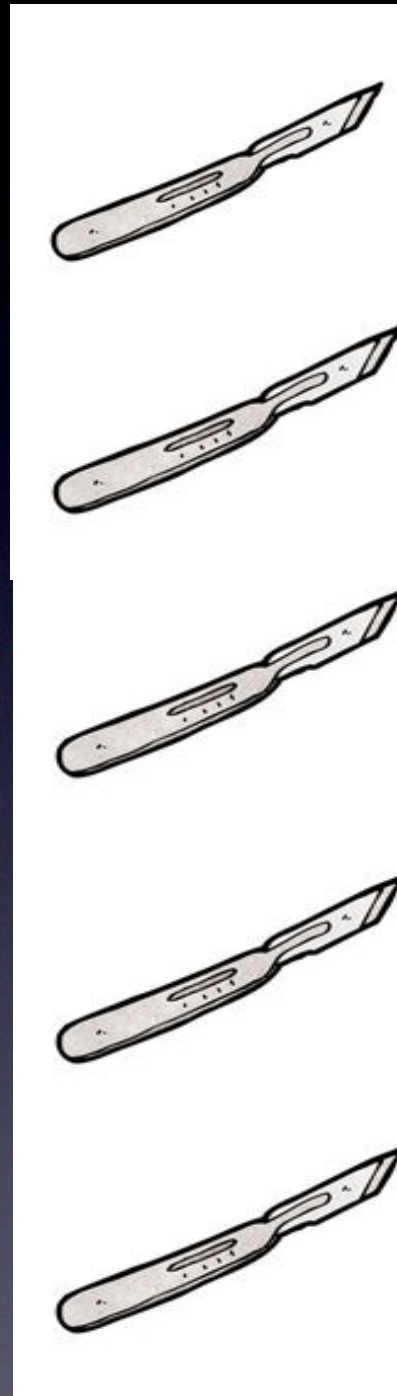
**ULCERE VENOSE CRONICHE E
POTENZIALE USO DELLE
CELLULE STAMINALI**

CELLULE STAMINALI - Risultati Pilota

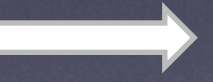
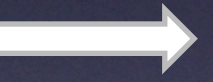
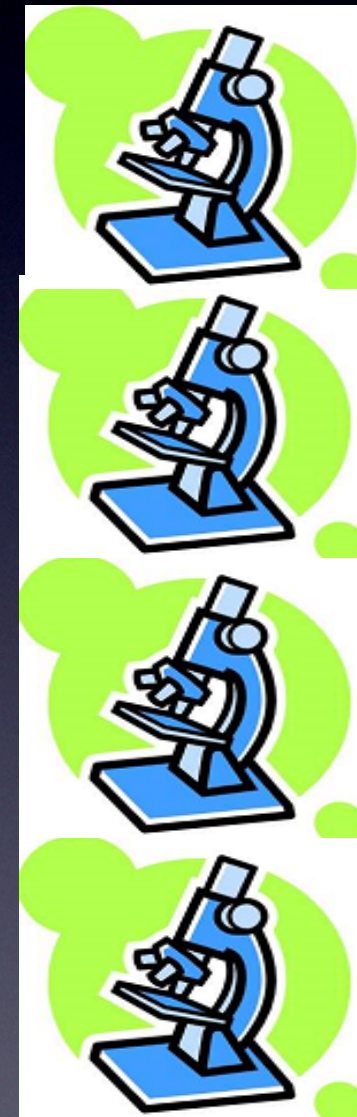
Investigators/ sponsor	Wound type	Cell type	Administration details	Number of patients	Phase	Outcomes
Badiavas et al. 2003 [53]	Various chronic wounds	Fresh BM aspirate Cultured adherent BM cells	Fresh BM aspirate injected into wound; 3 additional topical applications of cultured cells	3	Case studies	All wounds healed with 3 applications or fewer
Falanga et al. 2007 [55]	Acute surgical	Cultured and profiled BM-MSCs	Topically suspended in fibrin spray	5	Case studies	All acute wounds closed by 8 weeks
	Chronic lower limb			8		Chronic wounds healed faster with more cells
Yoshikawa et al. 2008 [56]	Intractable dermatopathies	Cultured BM-MSCs	MSCs on collagen sponges used as wound dressings	20	Case studies	Wound healed in 18 d the 20 patients
Dash et al. 2000 [57]	Nonhealing ulcer or lower extremities	Cultured autologous BM-MSCs	Single intramuscular injection and topical application on wound with standard wound care or standard wound care alone	24	Randomized controlled study	By 12 weeks: Improvement in pain- free walking Ulcer size decreased by 72% in MSC- treated group
Lu et al. 2011 [58]	Diabetic critical limb ischemia with foot ulcers	Cultured autologous BM-MSCs	Single intramuscular injection of cells or normal saline control	41	Randomized controlled study	Improvement in painless walking time in MSC-treate group
		Fresh uncultured BM-mononuclear cells				Fastest rate of healing in MSC treated group

Pazienti e metodi

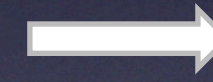
- B
- ML
- VAL
- MM
- BC



MARGOLIS



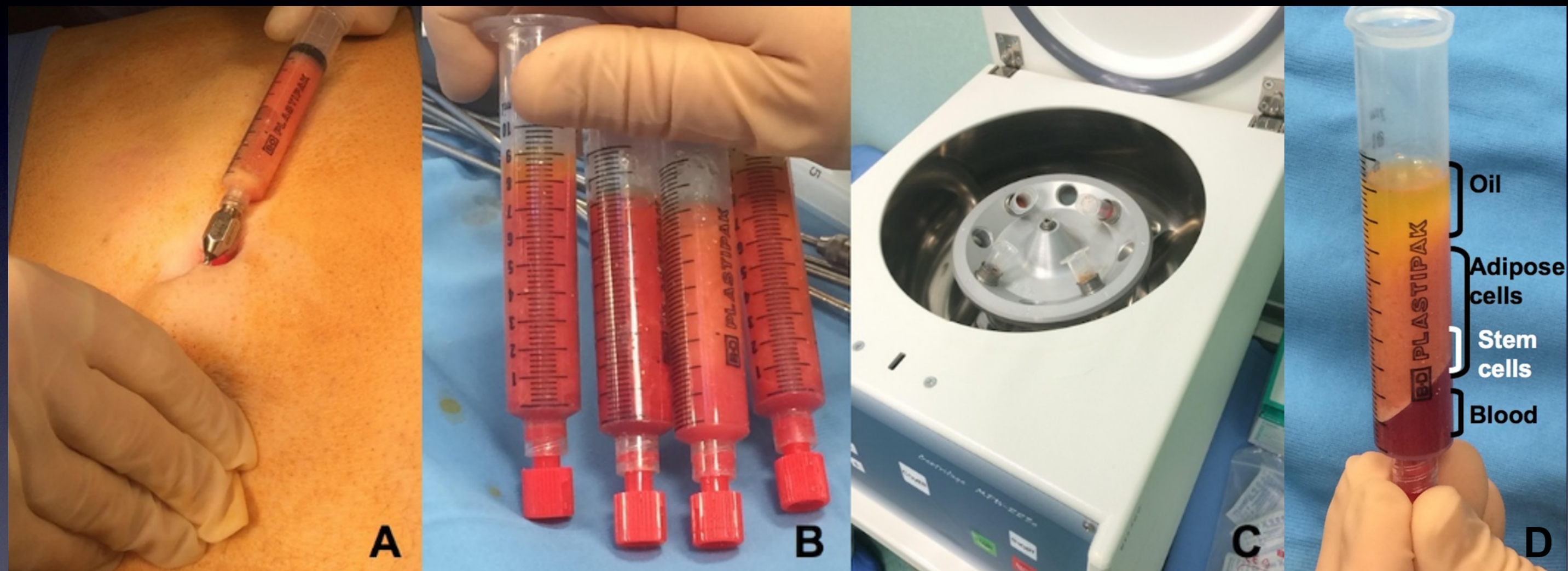
BIOPSIA



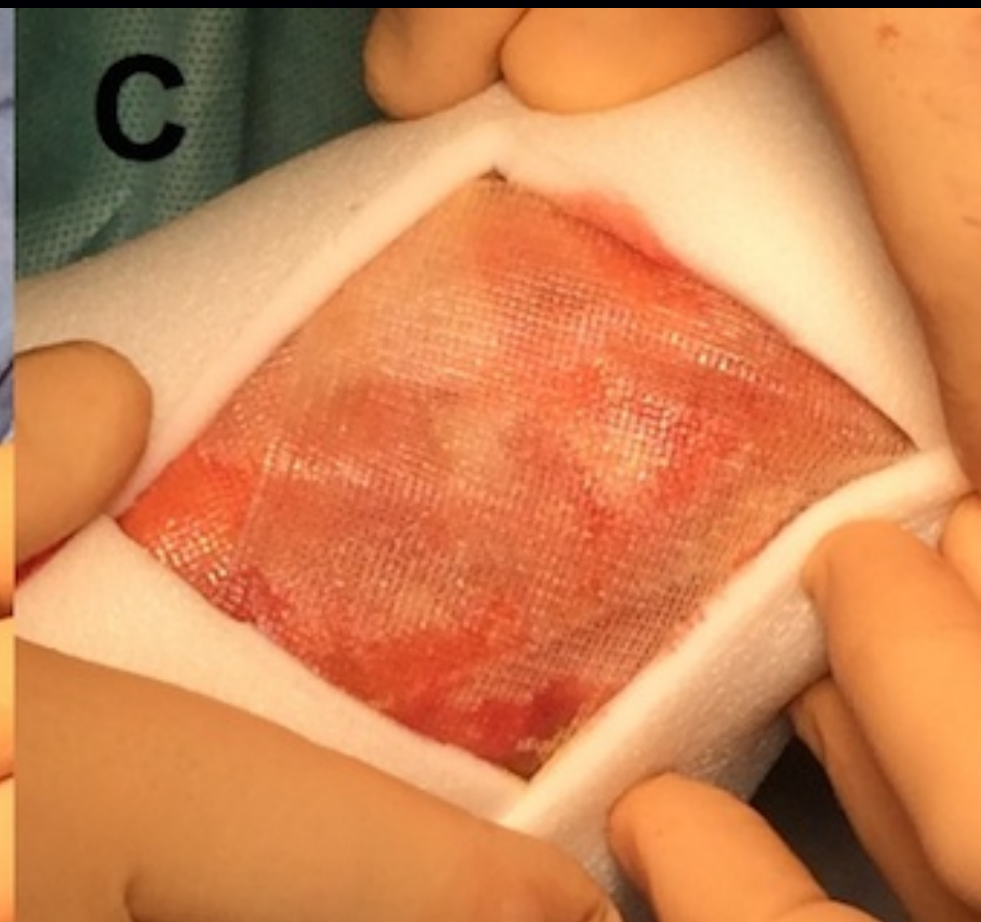
CITOLOGIA



Debridement chirurgico in anestesia locale

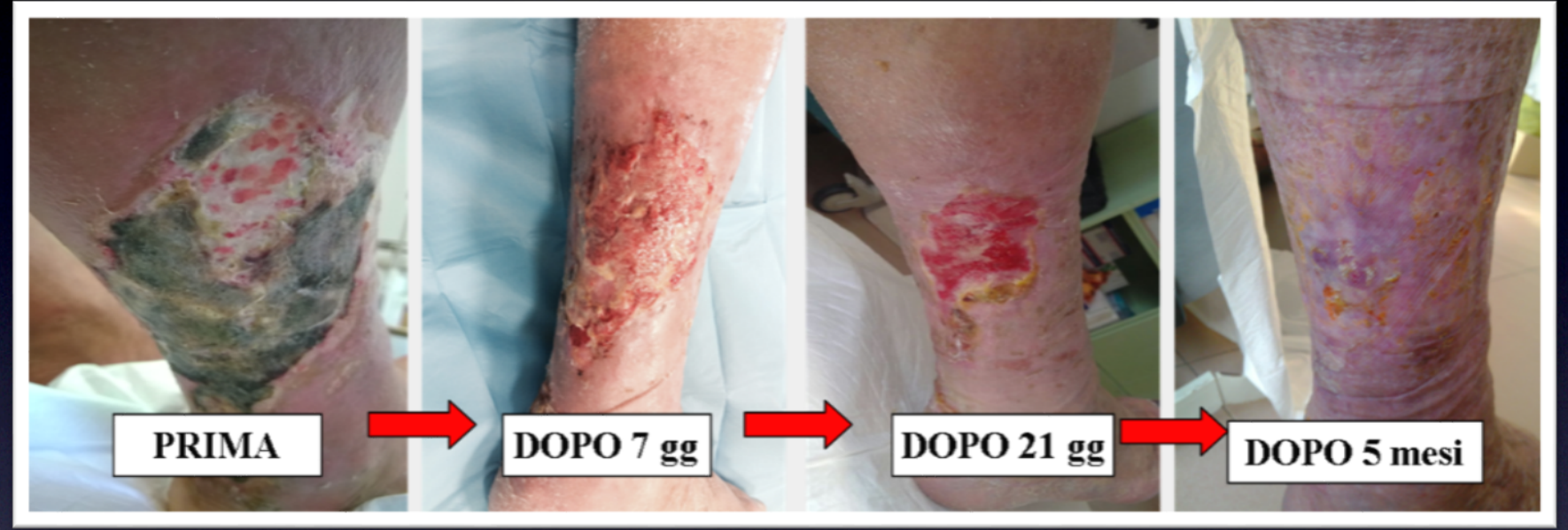
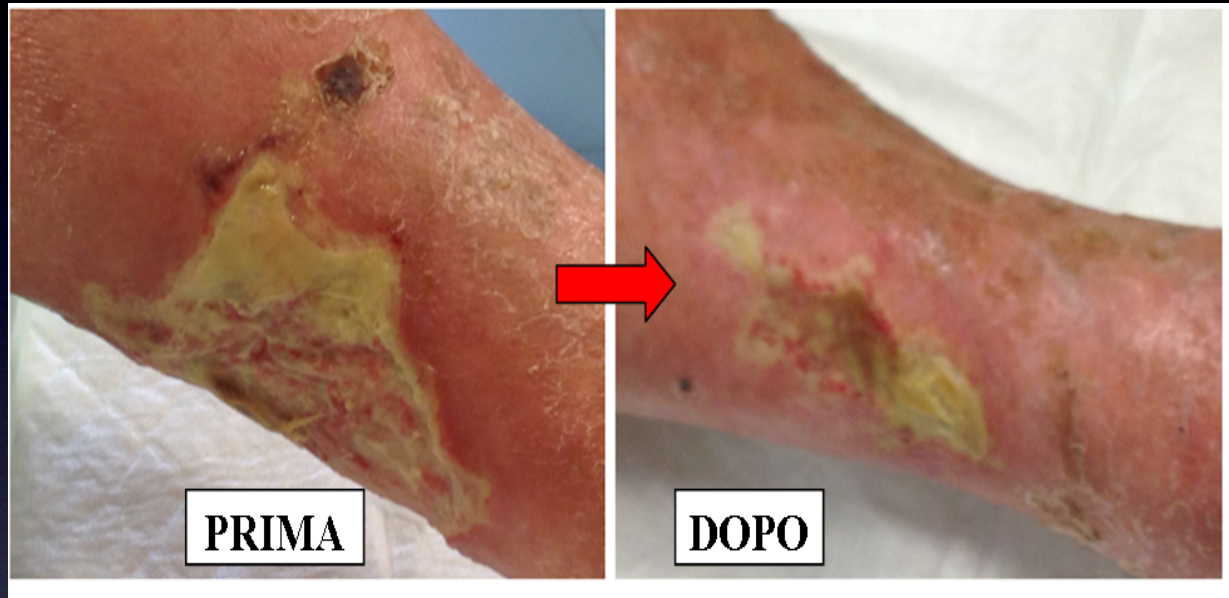


Prelievo e centrifugazione del tessuto adiposo



Innesto di cellule adipose nel letto della ferita e copertura con uno scaffold di acido ialuronico

Risultati Clinici



B

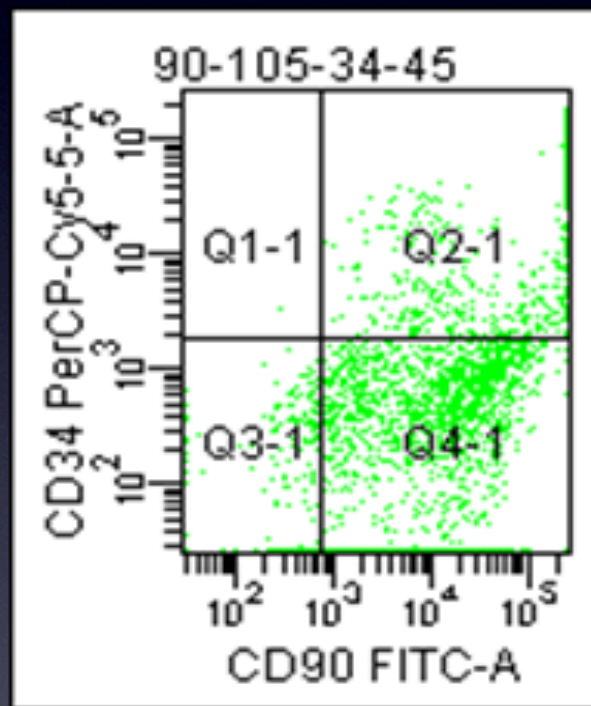
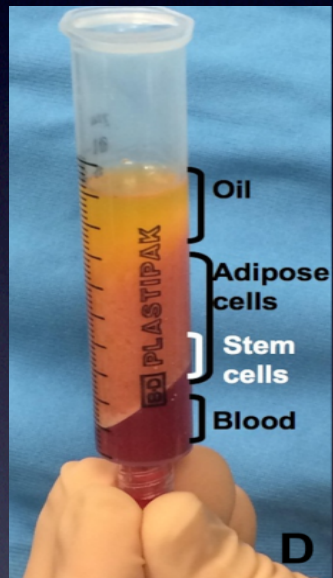
ML



VAL

CITOLOGIA

CARATTERIZZAZIONE CITOFLUORIMETRICA



PRIMA
della coltura

COLTURA

1. ENZIMATICO

EGM

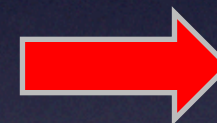
DOM

2. NON-ENZIMATICO

DOM

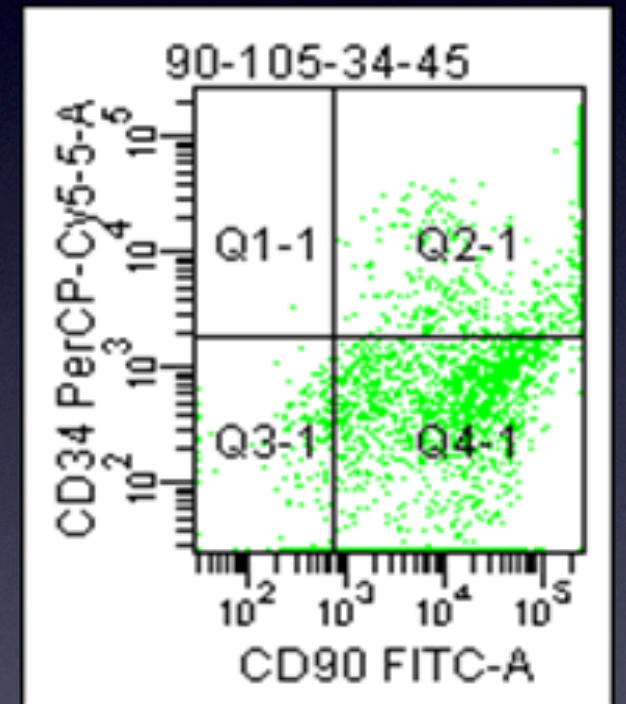
3. INTERO

DOM



NUM. FOCI

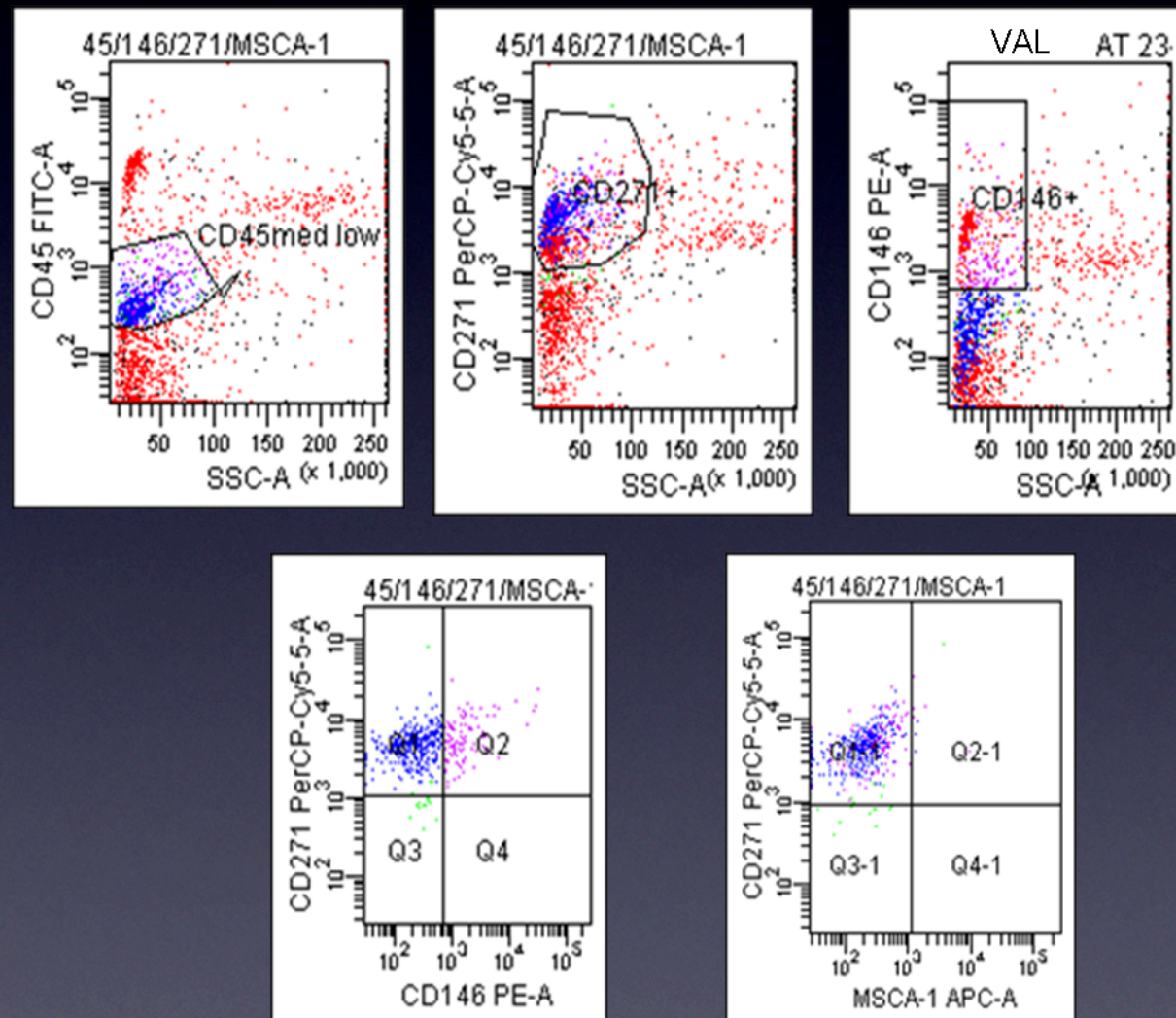
+



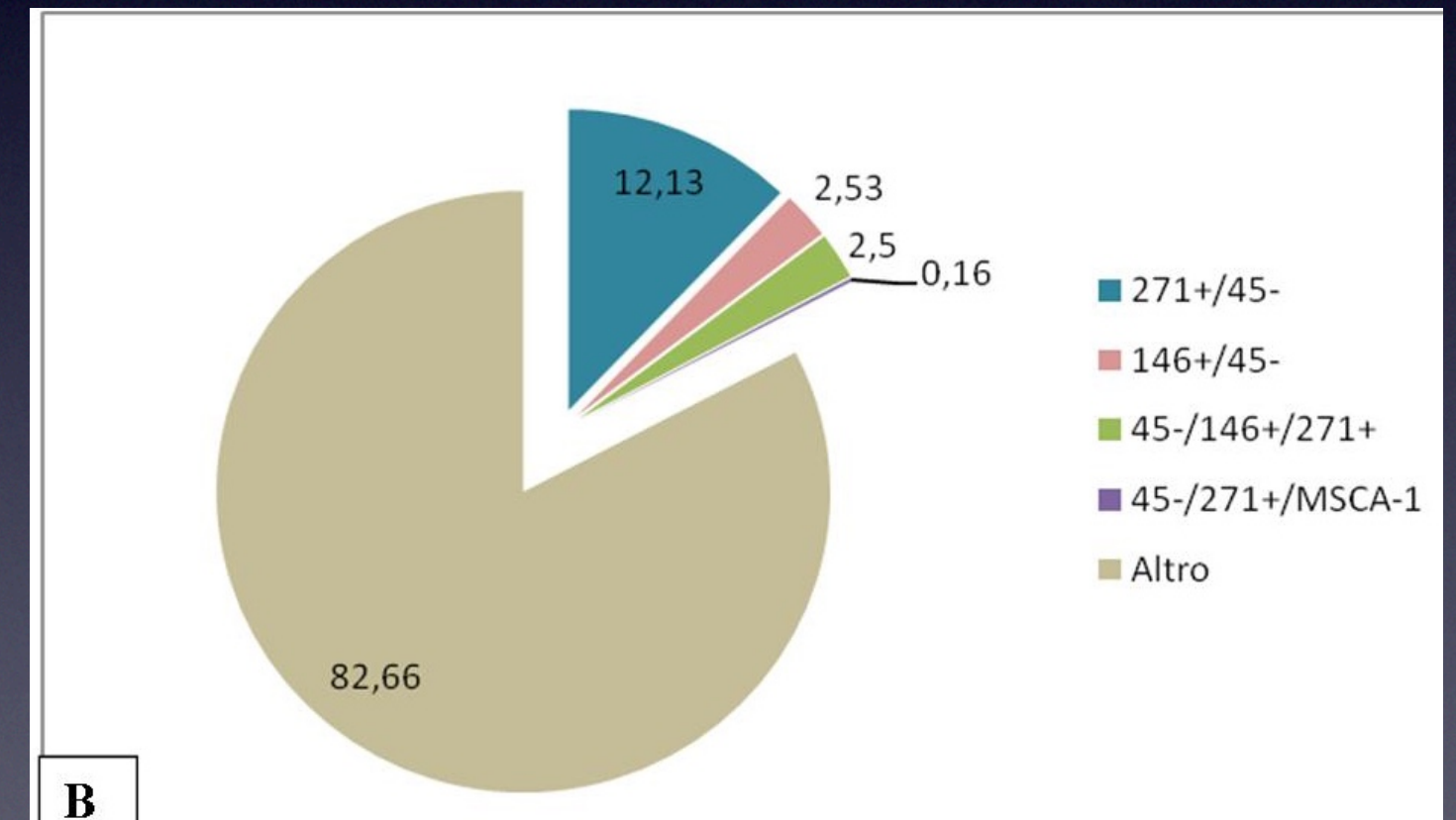
DOPO coltura

Risultati Citologia

PRE-COLTURA

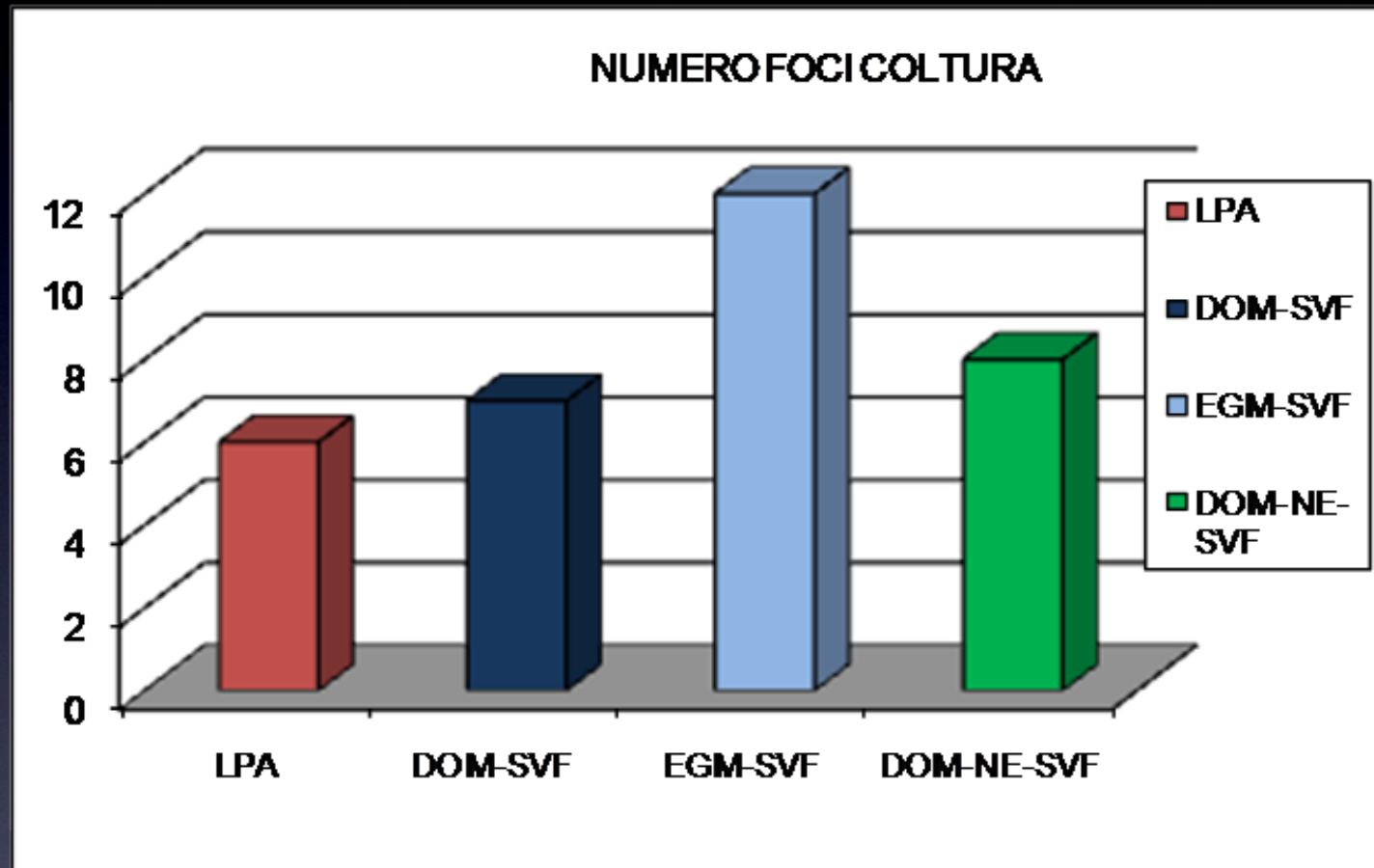


17% di CELLULE
MESENCHIMALI

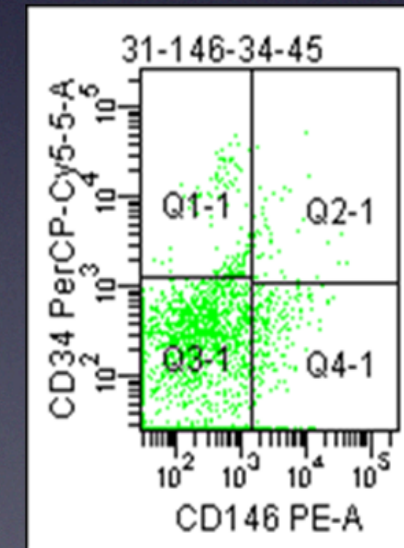
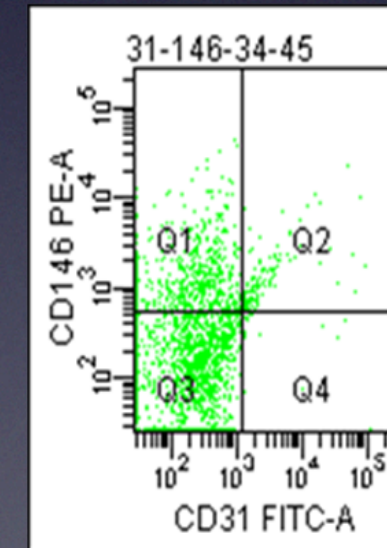
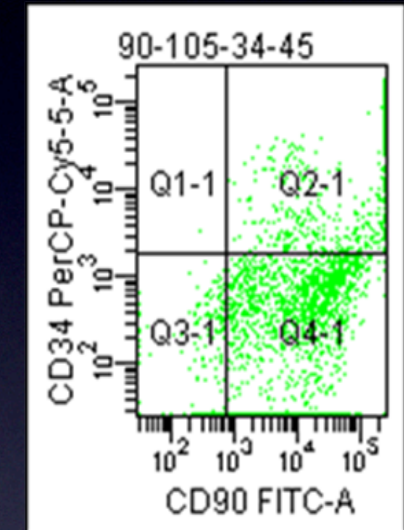
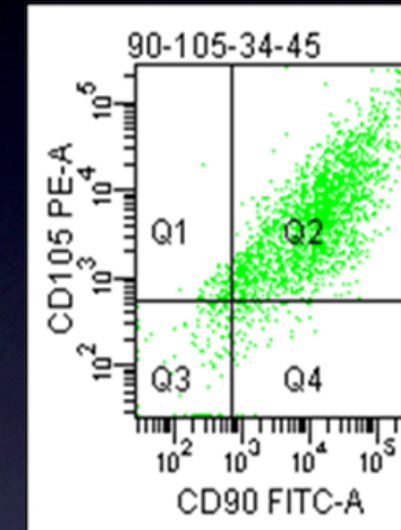
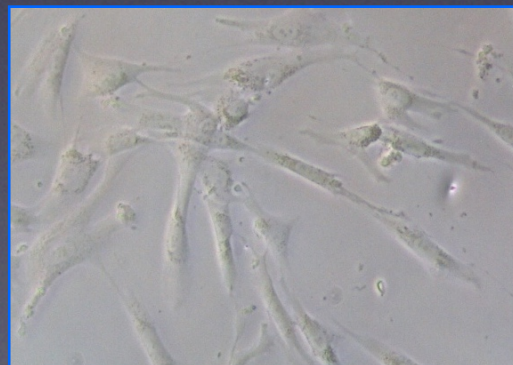
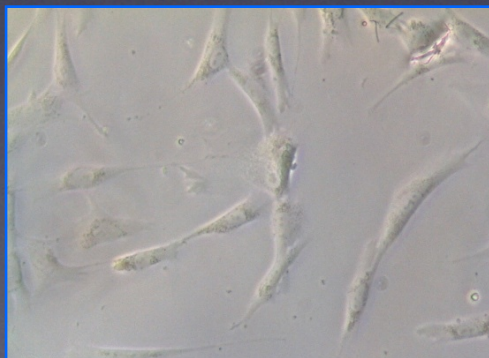


Risultati Citologia

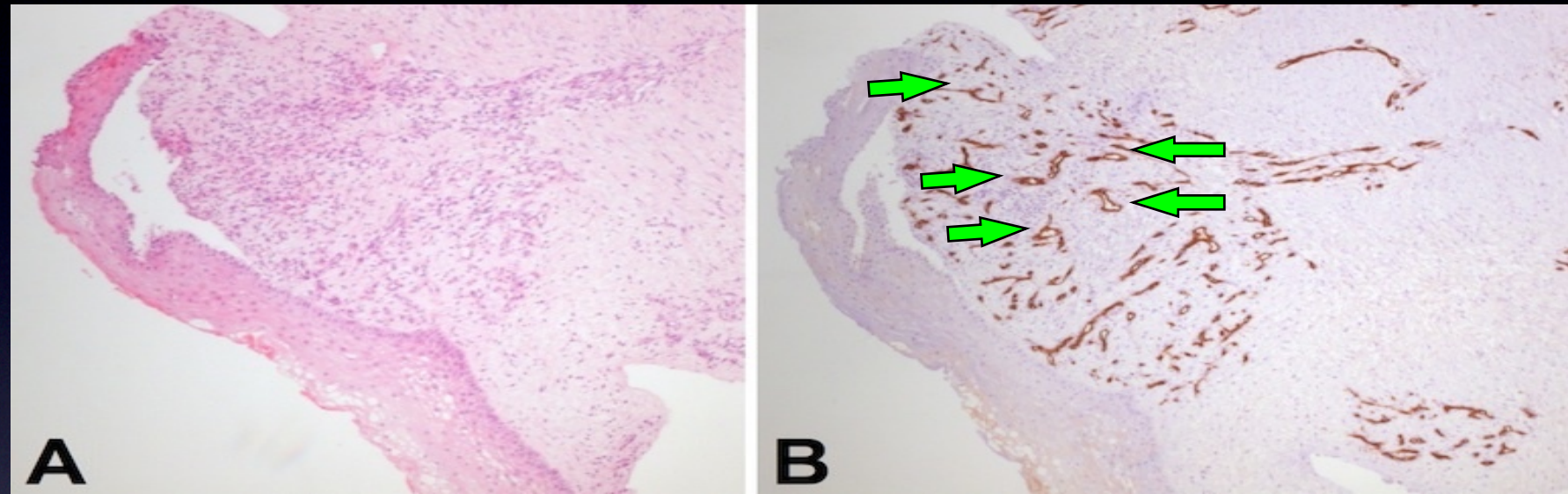
POST-COLTURA



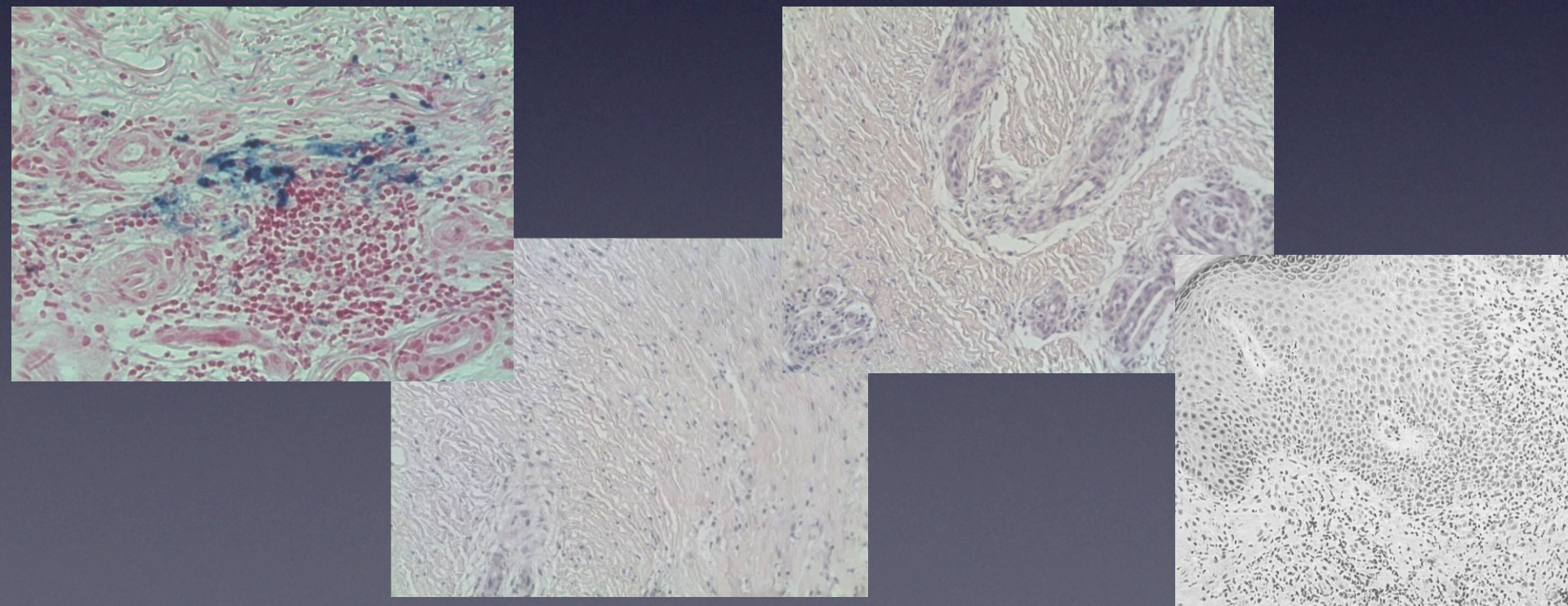
Crescita di
**CELLULE
MESENCHIMALI**



ANATOMIA PATOLOGICA



↑ ANGIOGENESI



INNESCO DEL
PROCESSO
RIPARATIVO

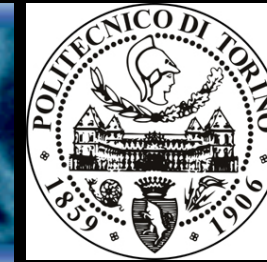
Proteine Rigeneratrici Sintetizzate dalle Staminali da tessuto adiposo

Specific proteins	Primary function
MMP1, MMP2, MMP3, MMP7, MMP8, MMP9, MMP10, MMP13 TIMP1 and TIMP2 Ang-2, HB-EGF, EGF, FGF-7 (also known as KGF), PlGF, PEDF, TPO, TGF- α , IGF bFGF; PDGF AA, AB, and BB; VEGF, VEGF-C, and VEGF-D	Matrix and growth factor degradation, facilitate cell migration Inhibit activity of MMPs, angiogenic Stimulate growth and migration Promote angiogenesis, also proliferative and migration stimulatory effects
TGF- β 3, HGF IFN- α 2 α 2-Macroglobulin	Inhibit scar and contracture formation Prevent fibrosis by decreasing TGF- β 1 and TGF- β 2 Inhibit protease activity, coordinate growth factor bioavailability
Acrp-30 IL-1RA N-GAL LIF SDF-1 β IGFBP1, 2, 3	Regulate growth and activity of keratinocytes Anti-inflammatory Antibacterial Support of angiogenic growth factors Recruit cells to site of tissue damage Regulate IGF and its proliferative effects

Abbreviations: Acrp-30, adiponectin; Ang-2, angiotensin-2; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; HB-EGF, heparin-bound epidermal growth factor; HGF, hepatocyte growth factor; IFN, interferon; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IL, interleukin; KGF, keratinocyte growth factor; LIF, leukemia inhibitory factor; MMP, matrix metalloproteinase; N-GAL, neutrophil gelatinase-associated lipocalin; PDGF, platelet-derived growth factor; PEDF, pigment epithelium-derived factor; PlGF, placenta growth factor; SDF, stem cell-derived factor; TGF- α , transforming growth factor- α ; TIMP, tissue inhibitor of matrix metalloproteinase; TPO, thrombopoietin; VEGF, vascular endothelial growth factor.

PROSPETTIVE FUTURE





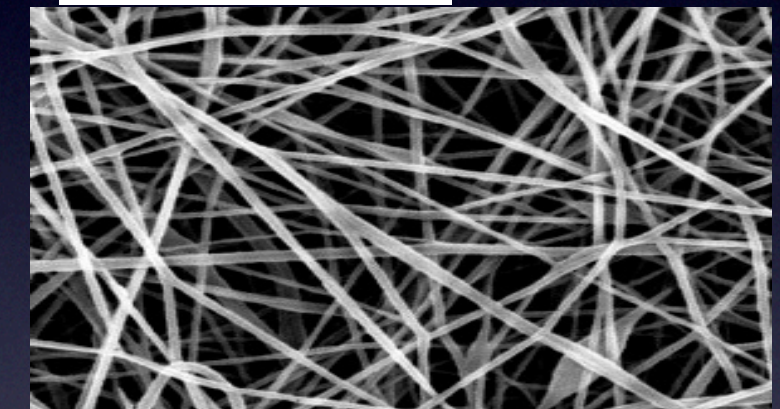
Materials for scaffolds.

- Collagen into which cells are cast or inserted
- Collagens modified by chemical cross-linking of the molecules
- Artificial molecularly engineered polypeptides
- Some polyaminoacids
- Hyaluronic acid and related compounds
- Agarose
- Alginates
- Poly-lactide fibre
- Poly-glycolide fibre
- Polydioxanone
- Polycaprolactone
- Polyvinyl alcohols (soluble slowly in body fluids)
- Non-degradable polymers such as polyethylene
- Silicones
- Hydroxyapatite (the bone mineral)
- Bioglasses, degradable or otherwise
- Metallic materials, usually titanium, some stainless steels

} these four are biodegradable polymers

Di Martino A, Sittinger M, Risbud MV. Chitosan: A versatile biopolymer for orthopaedic tissue-engineering. *Biomaterials* 26 (2005) 5983–5990.

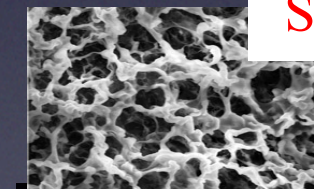
NANOFIBRE



FILM



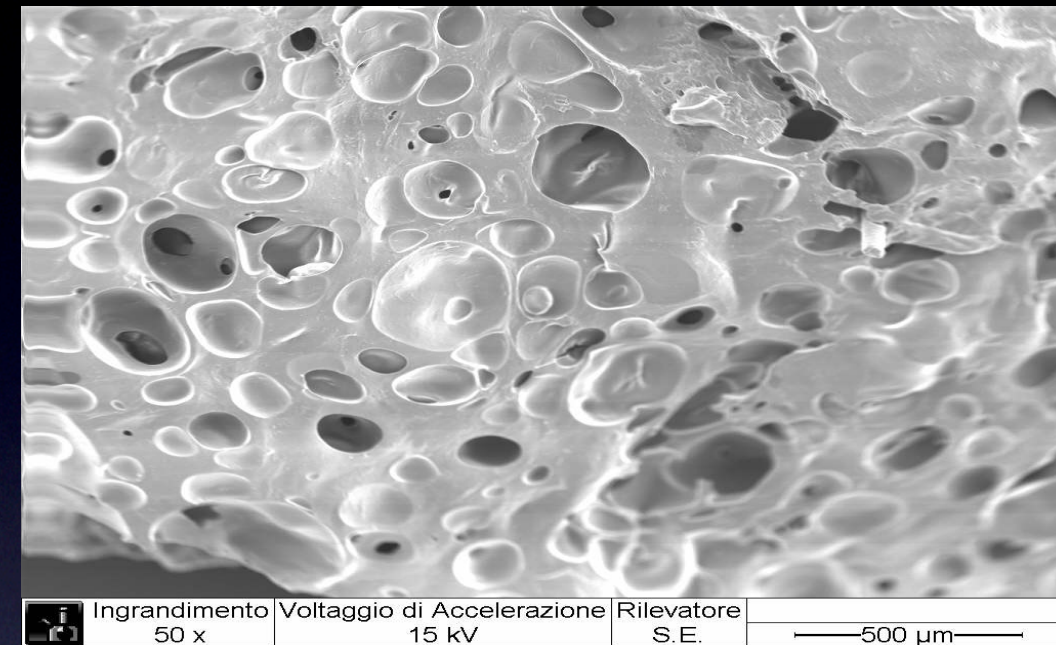
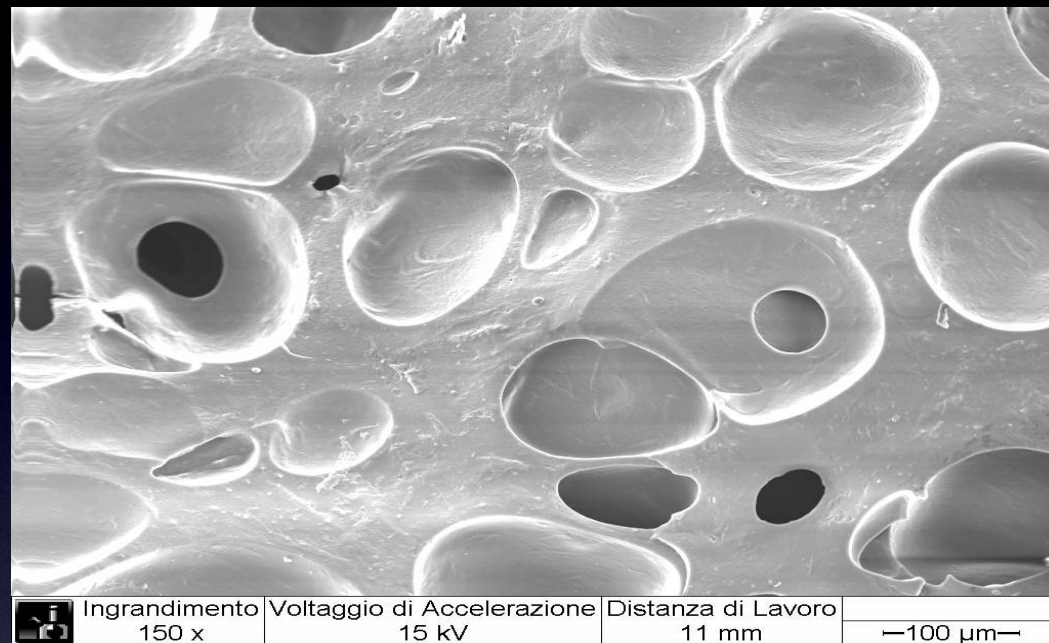
SPUGNE



IDROGEL

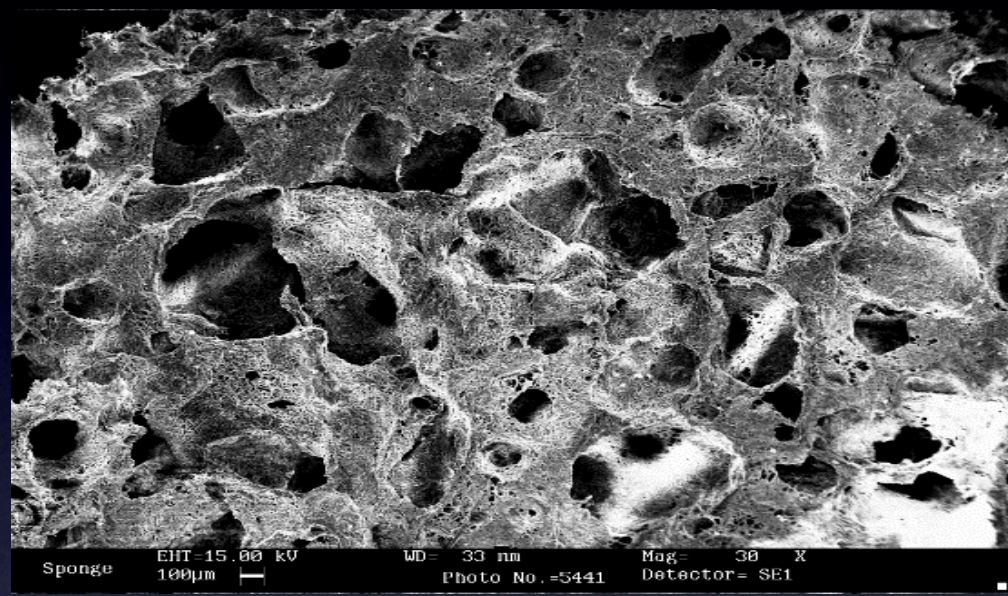


SCAFFOLD DI CHERATINA: I GEL



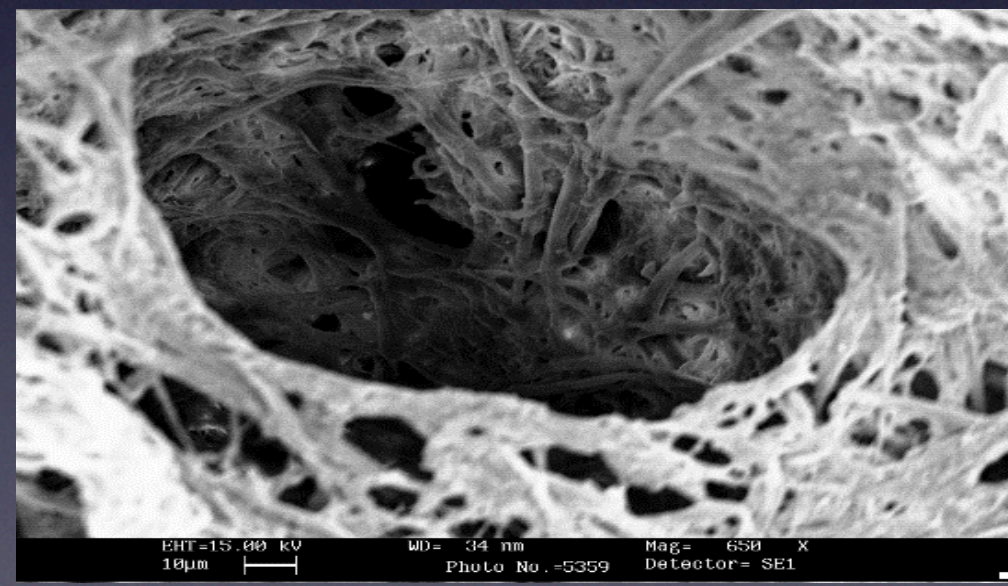
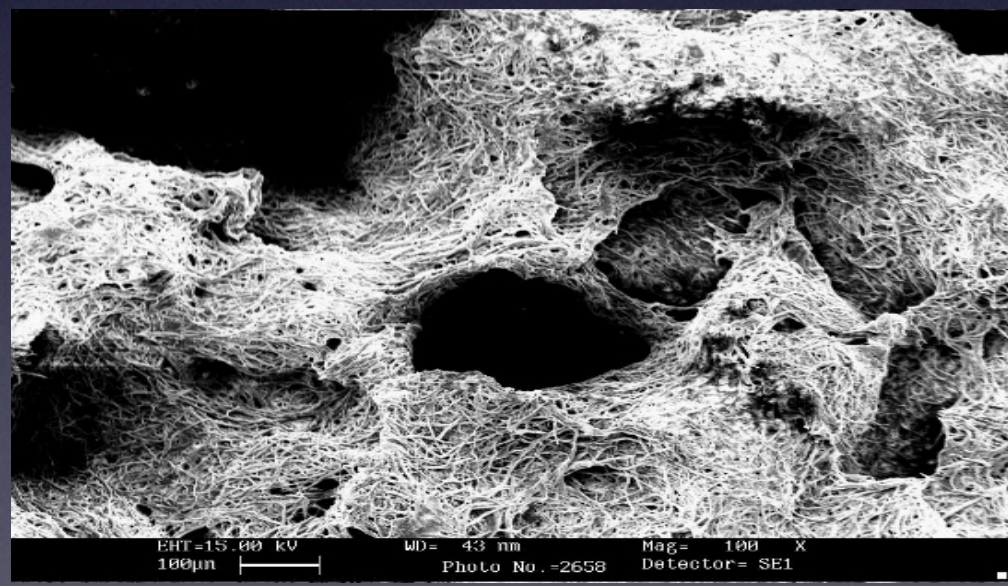
SCAFFOLD DI CHERATINA: LE SPUGNE

Porosità interconnessa:
93%
Densità vera: 1.32 g/cm³
Densità apparente di 0.09
g/cm³



Sezione

Dimensione delle cavità: 190-
560 µm
Media = 290 µm
Dev. St. = 110 µm



Morfologia delle spugne di cellule corticali - SEM

Quali i requisiti degli scaffold ideali nei processi di riparazione tissutale?

- Sterilizzabilità
- Elevata porosità
- Interconnessione tra i pori (contatto fra cellule e vascolarizzazione)
- Resistenza all'acqua, alle temperature
- Resistenza agli enzimi proteolitici
- Proprietà antimicrobiche
- Anisotropia
- Stabilità
- Biocompatibilità
- Elasticità (trasmissione di forza contrattile)

Obiettivo della ricerca

Modello animale  “*First in man*”



- Degradazione in vivo
- Reazione infiammatoria
- Tempo di riassorbimento