Seconda Università degli studi di Napoli

Azienda Universitaria Policlinico

UOC di Chirurgia Plastica e Ricostruttiva

Direttore: Prof. Francesco D'Andrea

Medicina Rigenerativa in Chirurgia Plastica
Dal trapianto di grasso all'ingegneria tissutale in vivo:
Nostre Esperienze

Prof. Francesco D'Andrea

16 aprile 2011 - Napoli

Un po' di storia…

innesto di tessuto adiposo

- **1893 Neuber :** ***innesto di adipe per correggere un difetto facciale***
  - → trapianto di piccoli pacchetti di grasso
  - → il tasso di successo dipende dalla grandezza dei frammenti
- **1910 Lexer :** ***depressione dopo frattura dell'arco zigomatico***
  - → trapianto di tessuto adiposo en block
  - → importanza di una tecnica atraumatica
- **1911 Bruning :** ***iniezione di grasso autologo nel sottocutaneo***
- **1926 Miller :** ***innesto solido di tessuto Adiposo con cannula***
- **1950 Peer : tasso di sopravvivenza del 50% nei trapianti di adipe**
  - → per la neo-vascolarizzazione necessari più di 4 giorni

Un po' di storia…

fat grafts or fat injection or lipofilling

- **1970 Fischer (ginecologo italiano): intuì la possibilità di "aspirare" il tessuto adiposo utilizzando una cannula ginecologica**
- **1977 Illouz : *The fat cell graft: una nuova tecnica per la correzione delle depressioni cutanee***
- **1981 Fournier : *micro-lipoestrazione e micro-lipoinjection per il ringiovanimento del volto (lipofilling de Fournier)***
- **1985 Vila Rovira: *tecnica trabecolare* mediante multiple incisioni cutanee intorno all'accumulo adiposo che consentono una tunnellizzazione crociata**
- **1986 Serra Renom: *dissezione-frammentazione-suzione* mediante la realizzazione di due incisioni a distanza, il suo scollamento ed infine l'aspirazione per frammentazione**
- **1986-2006 Coleman : *Sopravvivenza a lungo termine dell'adipe iniettato (lipostructure de Coleman)***

LIPOASPIRAZIONE

Regione donatrice

CENTRIFUGAZIONE
O
DECANTAZIONE

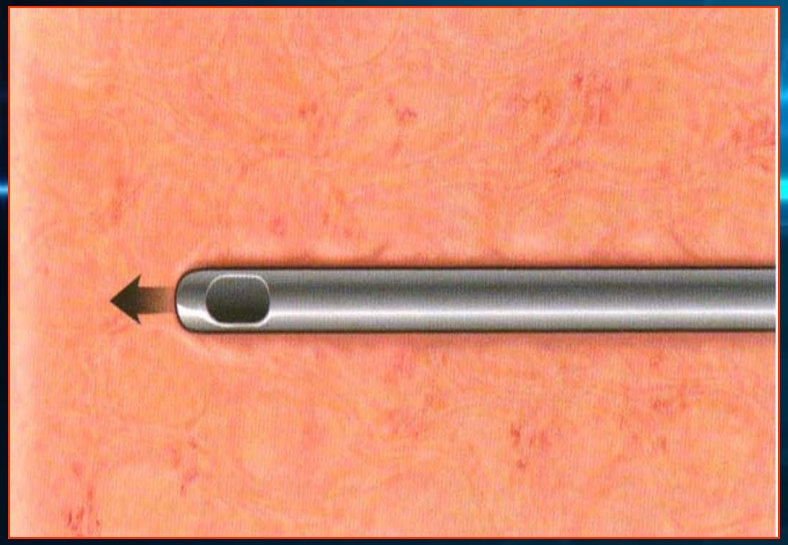
FRAZIONE
ADIPOCITICA

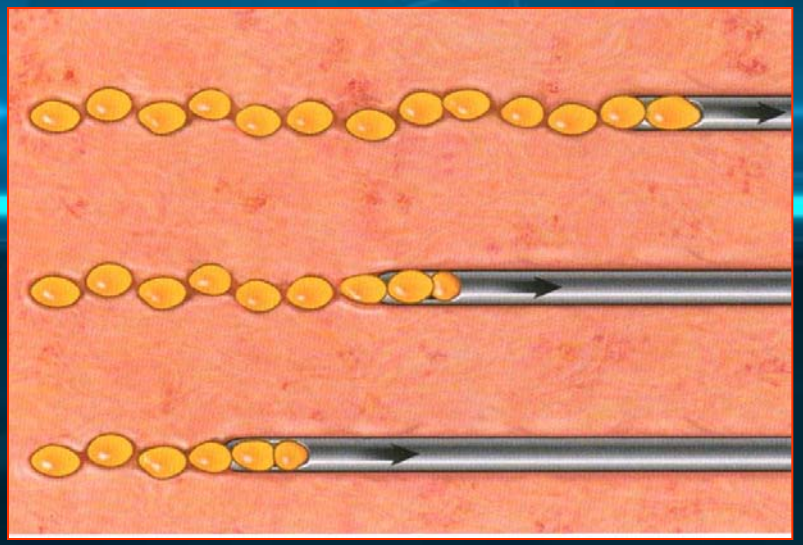
INNESTO

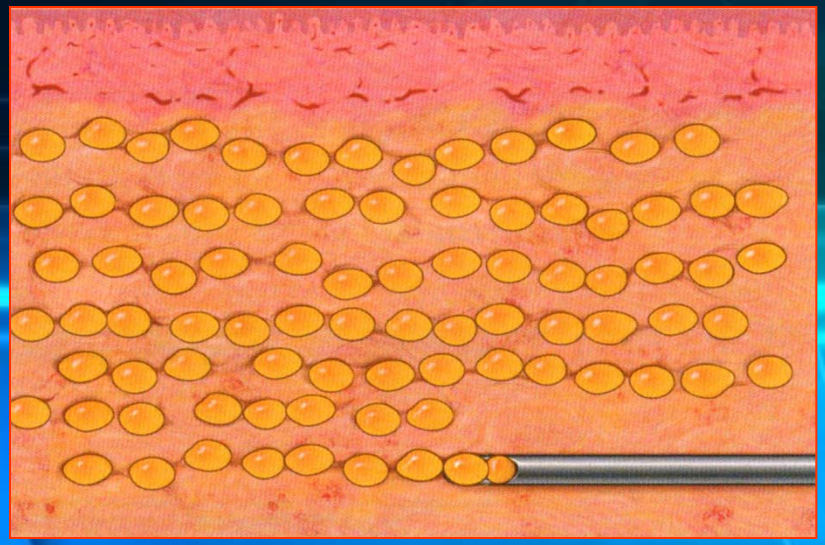
giunzione derma-ipoderma

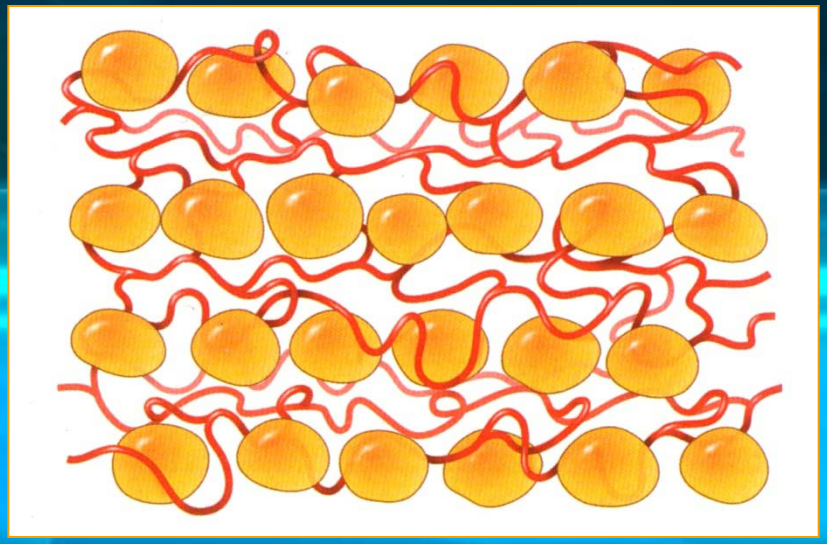
Aponeurosi – fascia muscolare

Lipostruttura



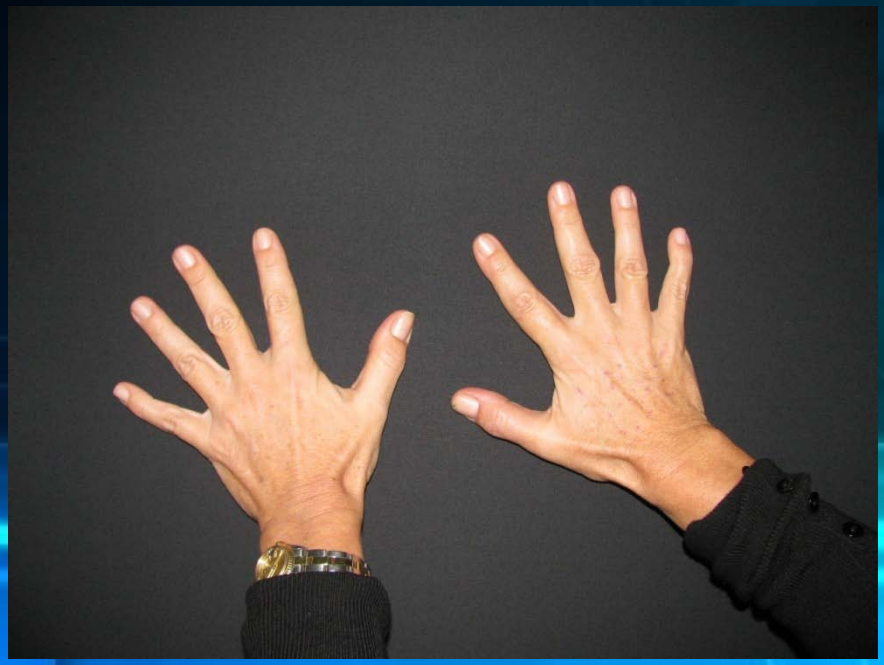


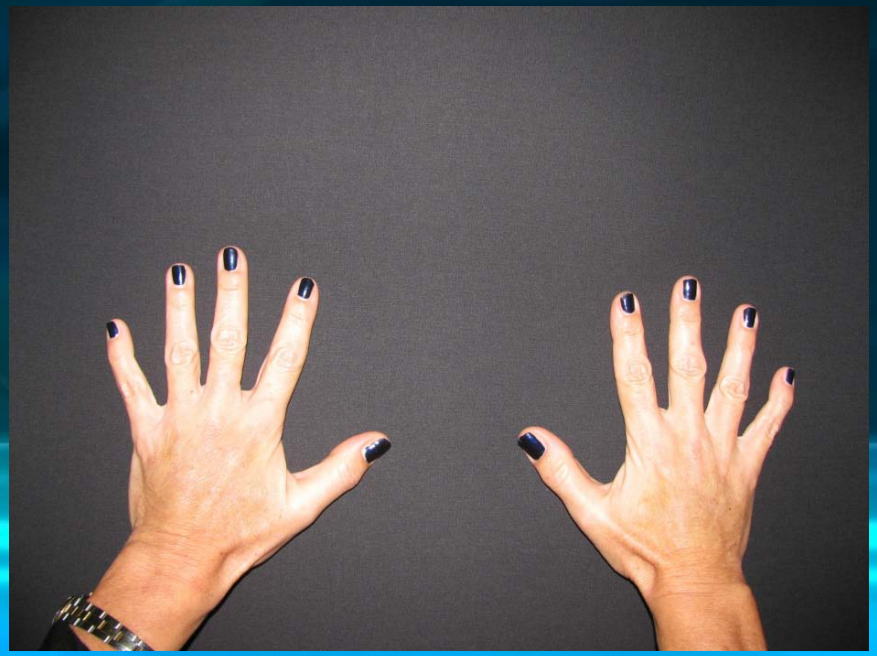




Caso clinico

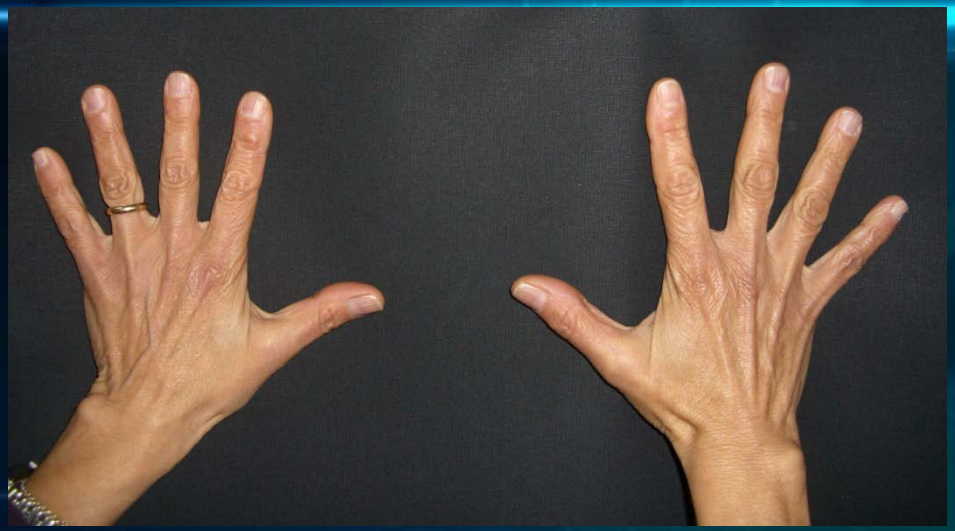
CHIRURGIA ESTETICA





Caso clinico

CHIRURGIA ESTETICA



Pre

Caso clinico

CHIRURGIA RICOSTRUTTIVA

Foto pre

Foto post




Autologous Fat Transplantation in plastic surgery:

“Filling Approach ”

from 1893

(Neuber et al. *Chir Kongr Verhandl Deisch Gesellsch Chir* ***22****, 66*)

Fat utilized as:

“Biocompatible Material”

Autologous Fat Transplantation in plastic surgery:

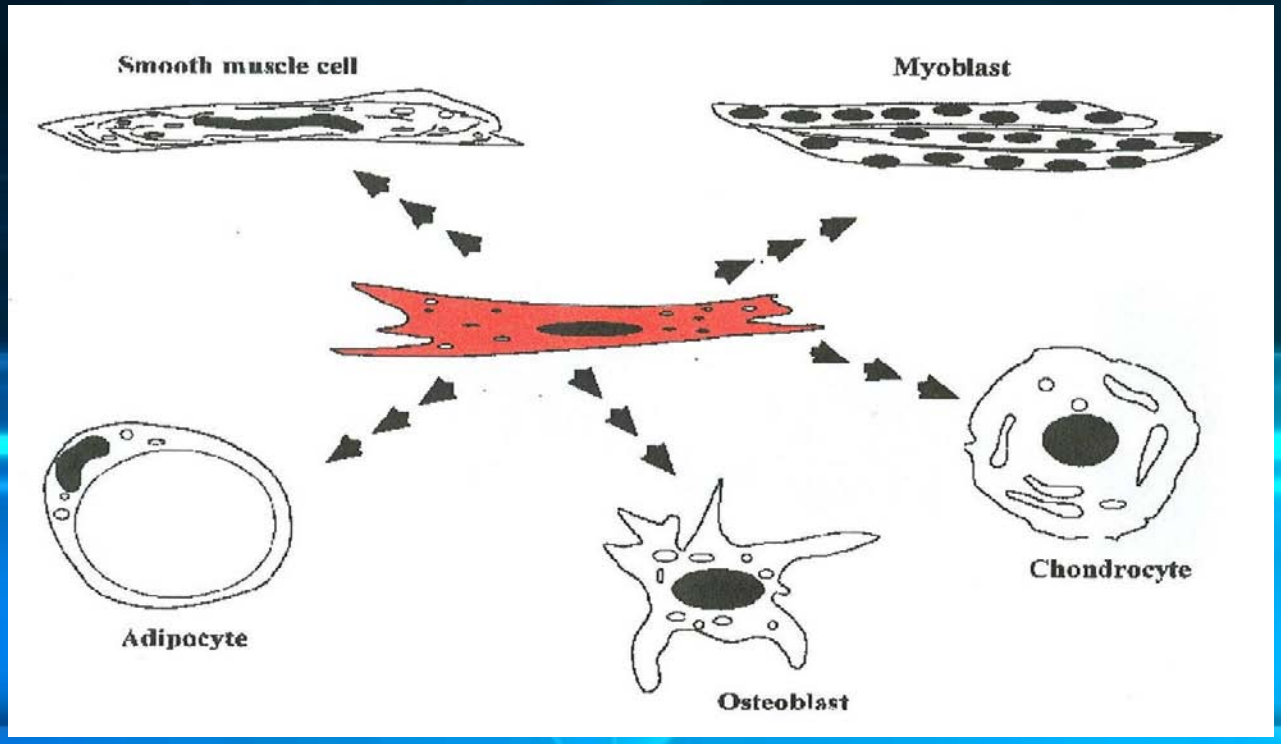
“Regenerative Approach ”

2007

Fat utilized as:

“Therapeutic Material”

I risultati del trapianto di grasso sono principalmente legati alla abbondante presenza delle cellule staminali adulte, cellule che hanno una straordinaria attività autorigenerativa e sono in grado di dare vita a diversi tipi cellulari e diversi tessuti.



The Era of Regenerative Medicine

“Tissue Engineering”

(1980’s - 2005)

Scaffold
Biomolecules
Cells
Tissues

“Regenerative Medicine”(2005)

Stem Cell
Materials
Biomolecules
Tissues

Objective:

So what’s of new?

Make the tissue

Repair the tissue

Tessuto Adiposo

The Tissue Engineering (regeneration) Approach

Key stages

Basic

Cells

-Source

-Differentiation

-Purification

Materials

Pre-clinical

Clonal expansion

In vitro function

3D Configuration

Animal models

Clinical

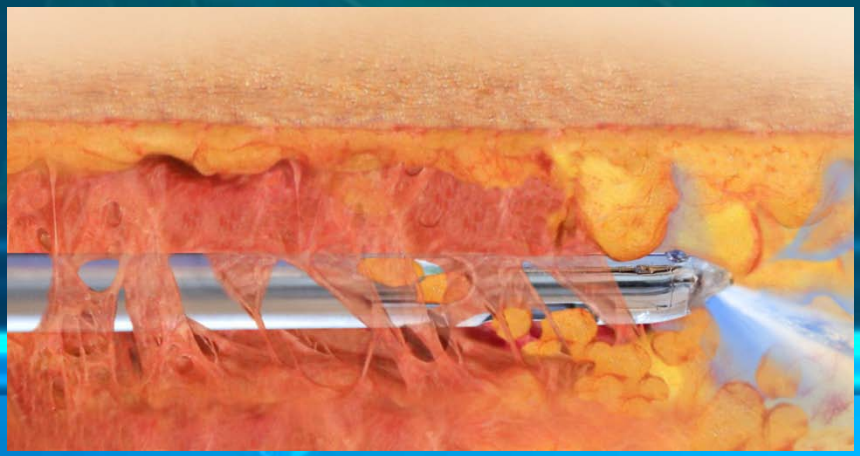
Cells therapy

Tissue regenerative

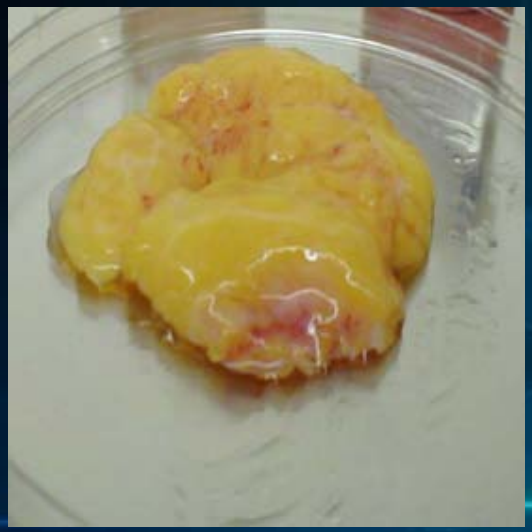
fase 1

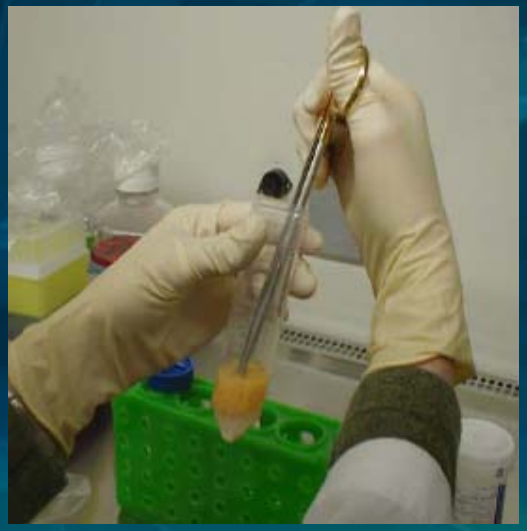
- Raccolta del tessuto adiposo
- Isolamento, purificazione ed espansione delle cellule staminali adipose
- Differenziazione multilineare con mezzi specifici

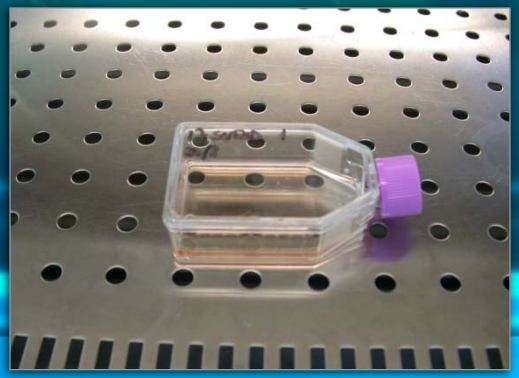
Tecnica WAL
Water Assisted liposuction

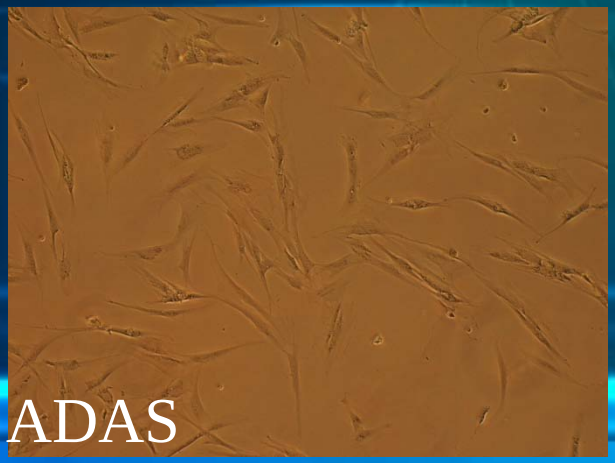


Tessuto Adiposo: estrazione, digestione e coltura cellulare









FACS Analysis

c-kit^{+}/ CD34^{+}/ CD45^{-} → CELLULE STAMINALI STROMALI

CD34^{+}/ CD90^{+}/ CD45^{-} → CELLULE STAMINALI STROMALI DALLA FRAZIONE VASCOLARE

CD44^{+}/ CD54^{+}/ CD45^{-} → ENDOTELIOCITI DALLA FRAZIONE VASCOLARE

Adult stem cell plasticity

Queste cellule sono “***committed, but not restricted***” ovvero *parzialmente* determinate ma *non strettamente* vincolate verso un unico destino.

Cellule coltivate in specifiche condizioni di differenziamento

- **Mezzo adipogenico**

Dexamethasone

Insulin

Indomethacin

IBMX

- **Mezzo Osteogenico**

Dexamethasone

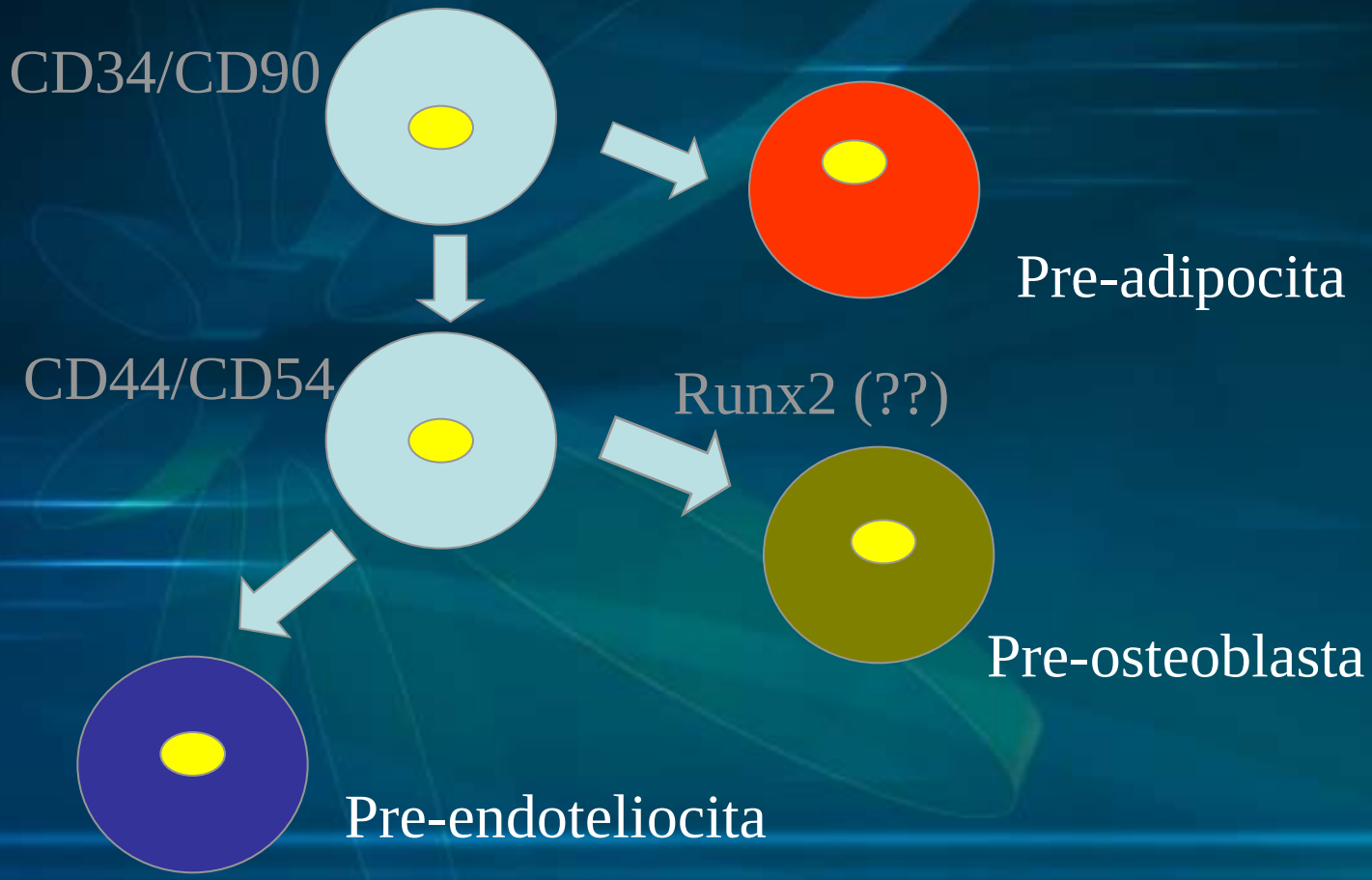
Ascorbate phosphate

B glicerophosphate

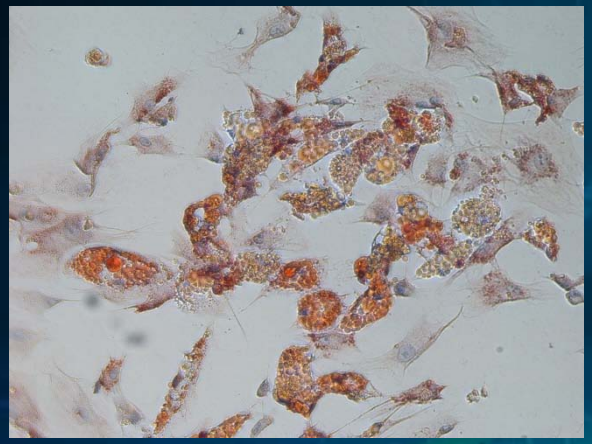
- **Mezzo Angiogenetico**

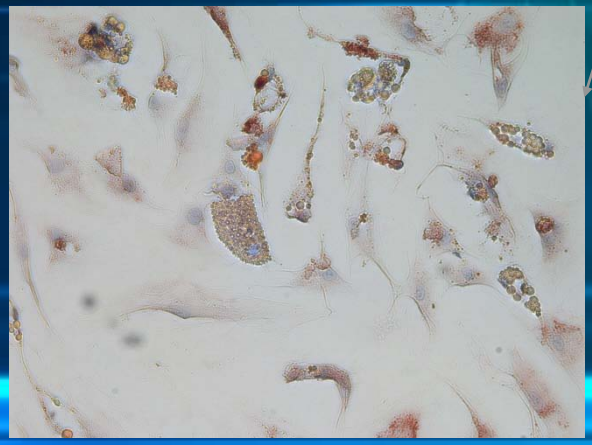
VEGF

Co-Differenziamento



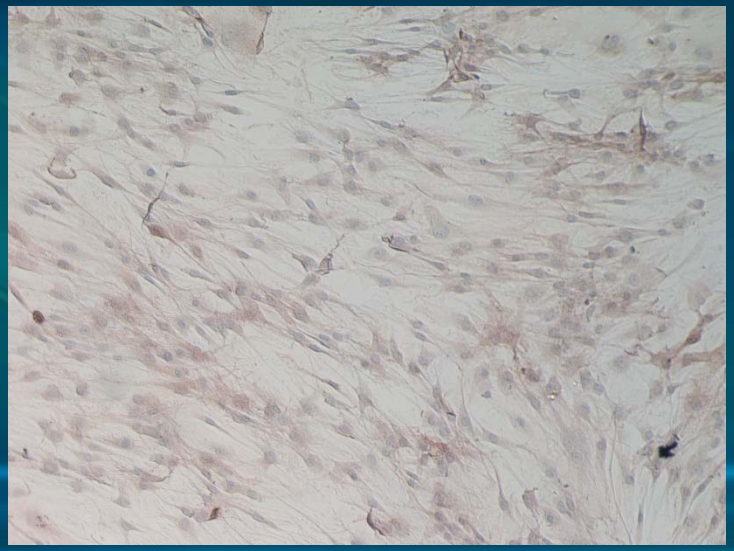
Differenziamento Adipogenico





$34^+/90^+$ cells in adypogenic medium

$34^+/90^+$ cells in DMEM

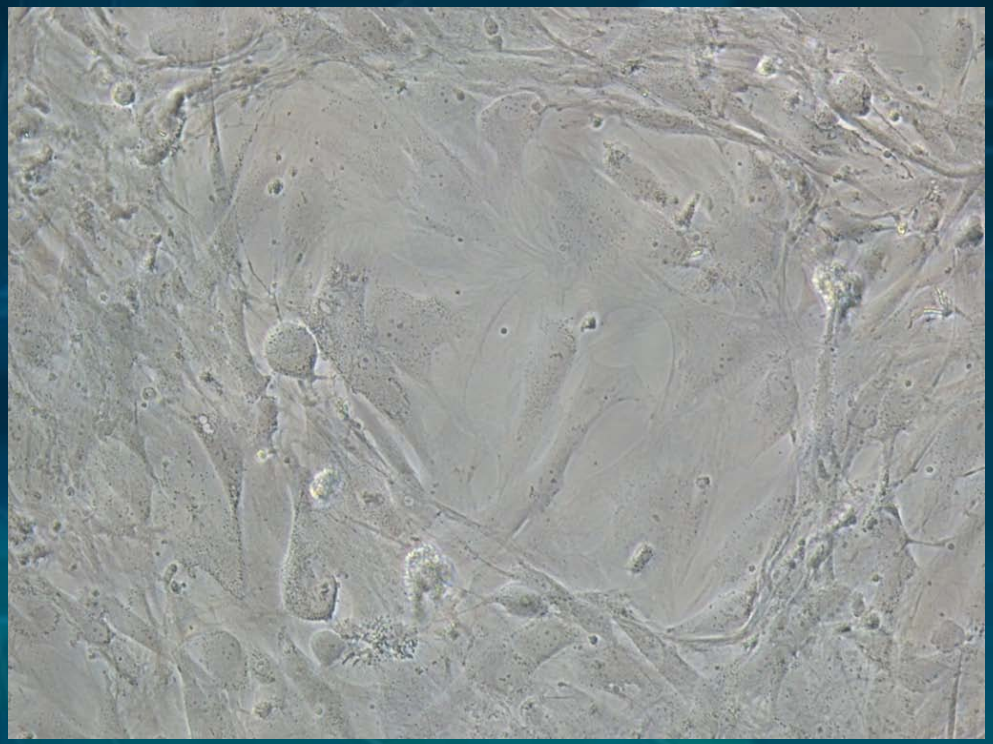


Differenziamento vascolare

CD 34

CD 133

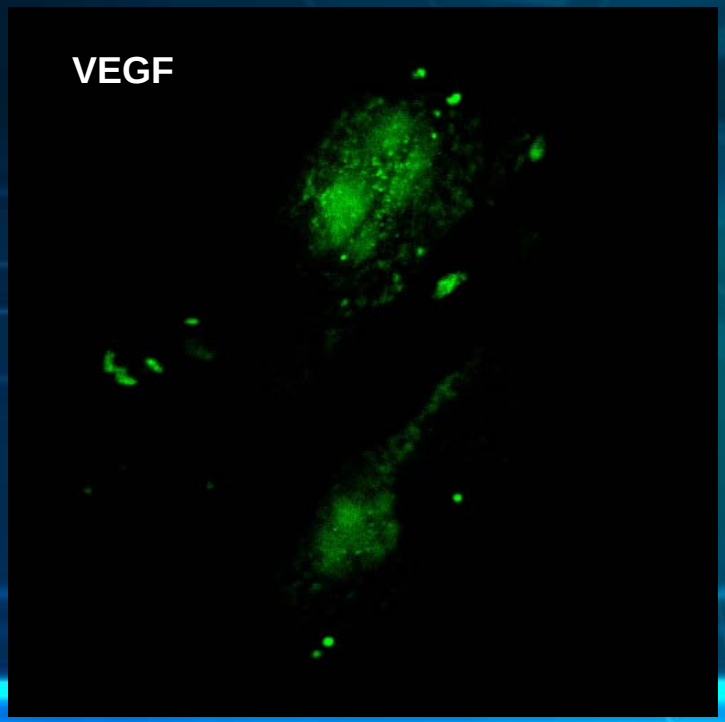
Flk-1

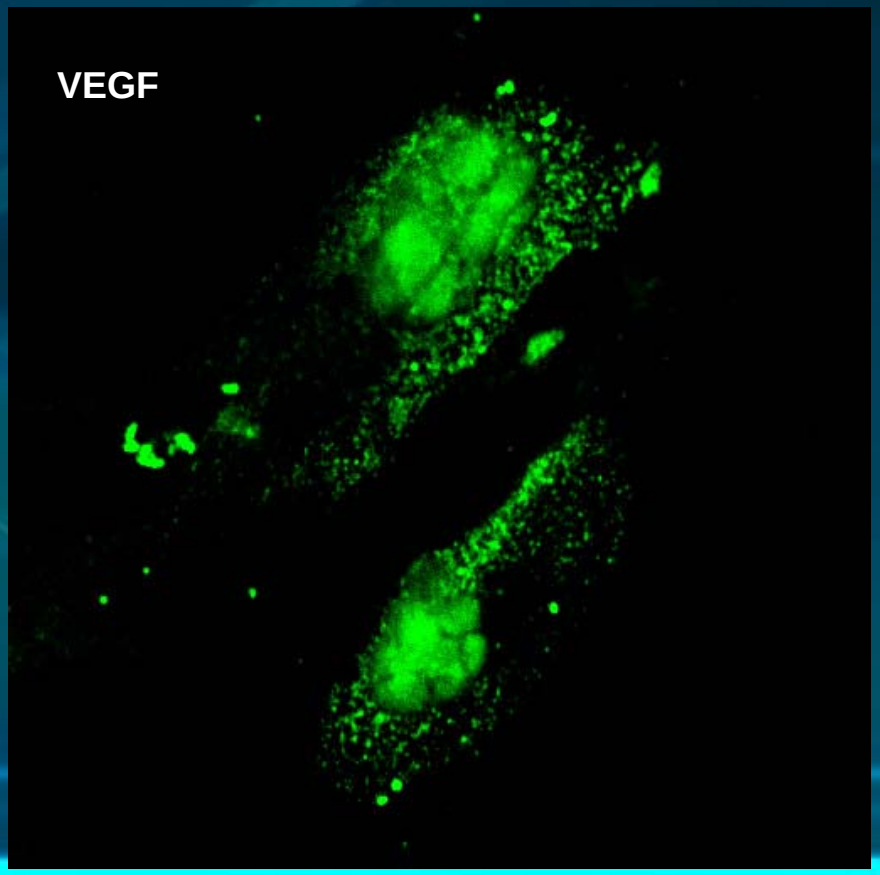


Le cellule in coltura, “polarizzano” formando una struttura simile ai vasi sanguigni

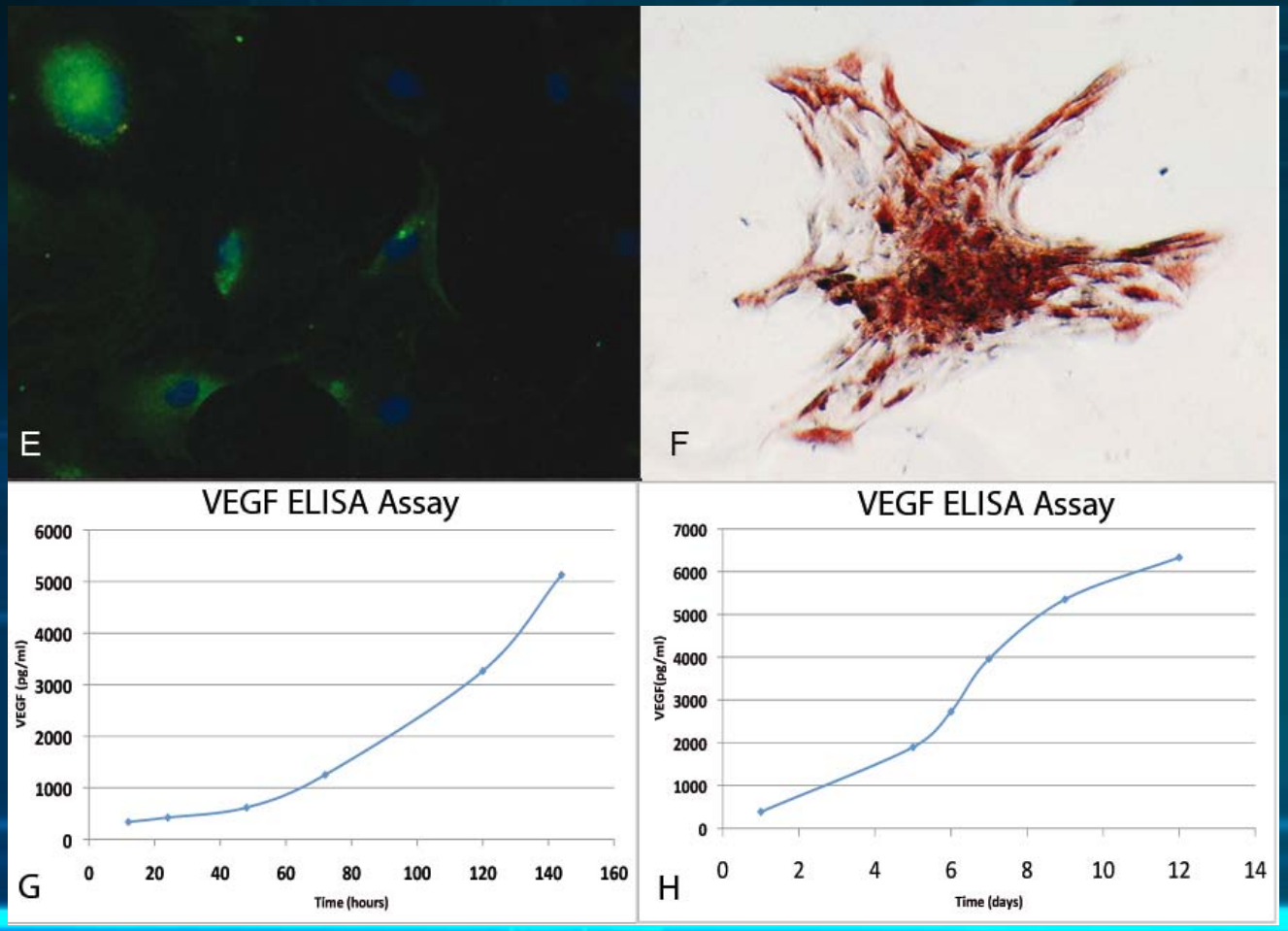
LAB VESSEL

Cells sorted for CD34/CD90 after 30 days





LAB VESSEL


E
F
VEGF ELISA Assay
VEGF (pg/ml)
6000
5000
4000
3000
2000
1000
0
0
20
40
60
80
100
120
140
160
Time (hours)
G
VEGF ELISA Assay
VEGF(pg/ml)
7000
6000
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
14
Time (days)
H

fase 2

- Riproduzione del Tessuto Adiposo “in vitro”

Differenziamento

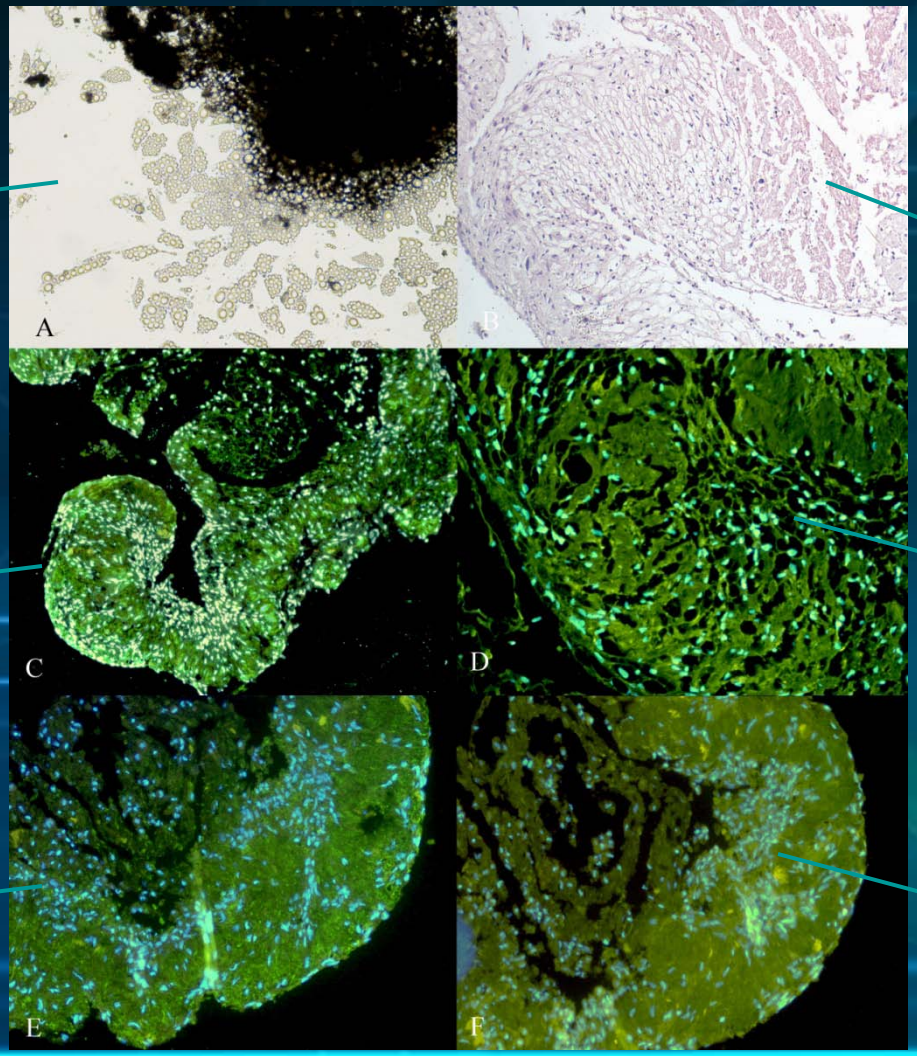
FBS 20% 3-5 months

DIFFERENTIATION

“IN VITRO” TISSUE

DEVELOPMENT

Neoformazione di Tessuto Adiposo *In Vitro*



Tessuto in coltura

Sezione in paraffina

IF per collageno di tipo I

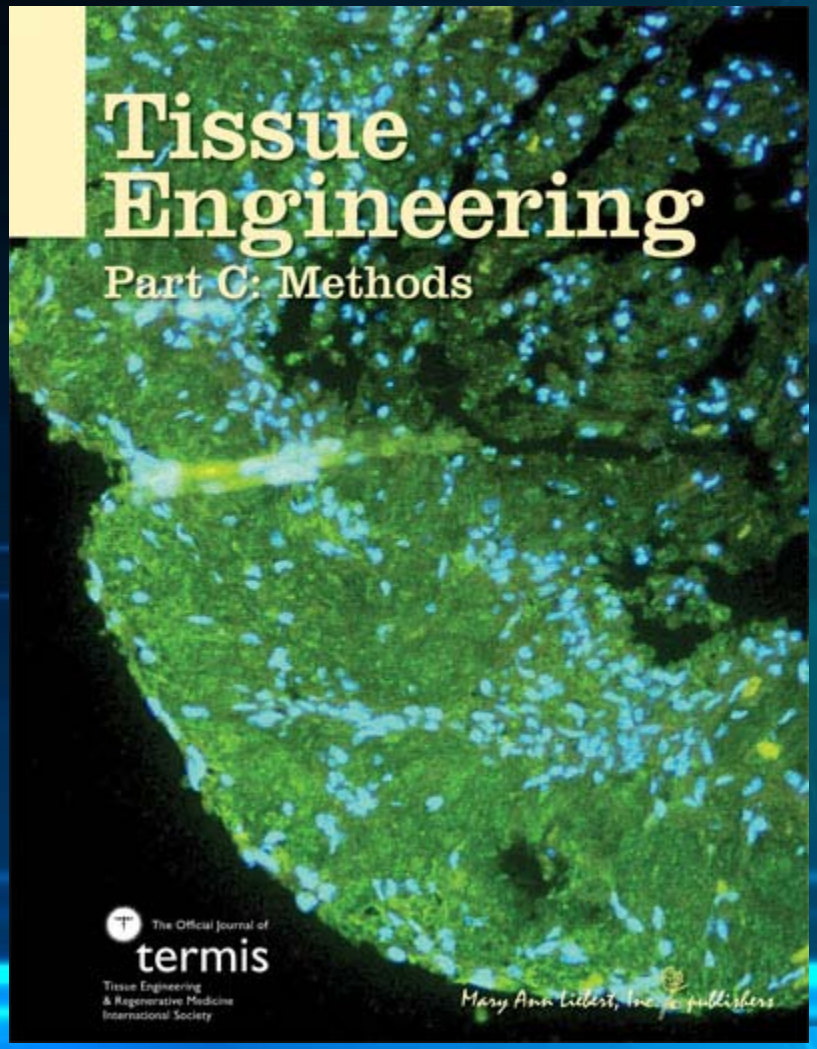
IF per fibronectina

IF per VEGF

IF per CD31

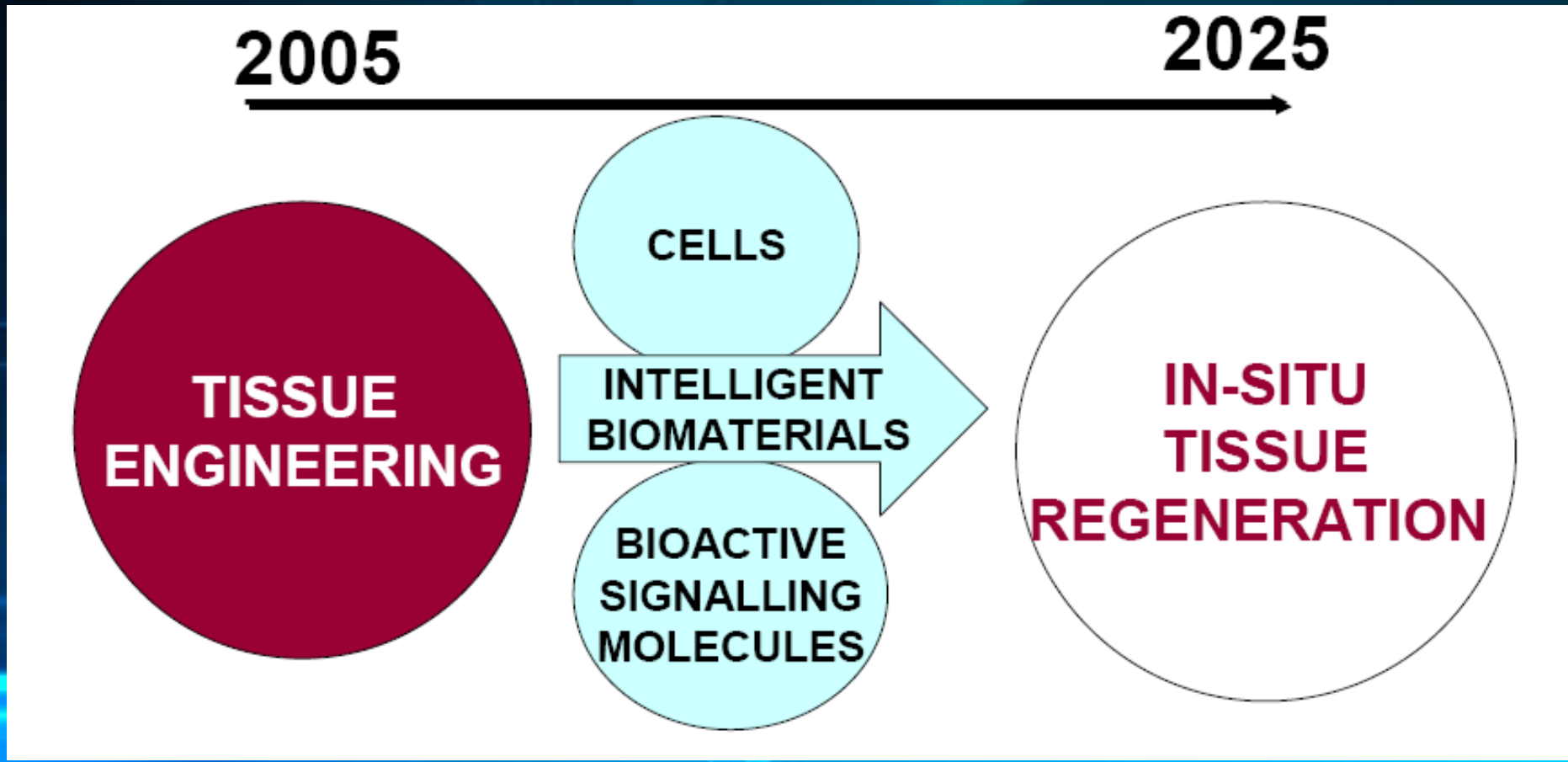
D'Andrea F, De Francesco F, Ferraro GA, Desiderio V, Tirino V, De Rosa A, Papaccio G.
Large-scale production of human adipose tissue from stem cells: a new tool for regenerative medicine and tissue banking.
Tissue Eng Part C Methods. 2008 Sep;14(3):233-42.

Tissue Engineering

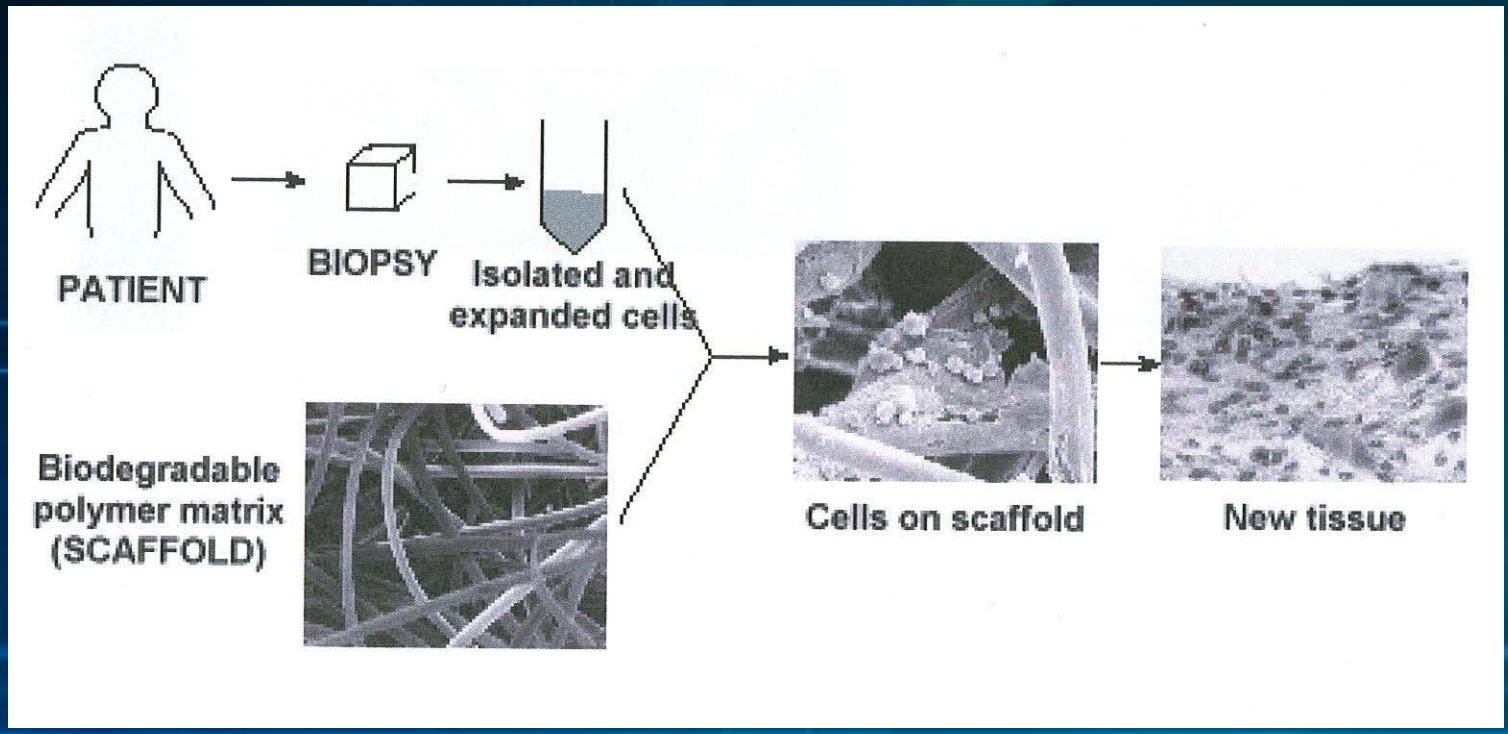


D'Andrea F, De Francesco F, Ferraro GA, Desiderio V, Tirino V, De Rosa A, Papaccio G.
Large-scale production of human adipose tissue from stem cells: a new tool for regenerative medicine and tissue banking.
Tissue Eng Part C Methods. 2008 Sep;14(3):233-42.

Fase 3
Ingegneria Tissutale
(riproduzione 3d del tessuto)

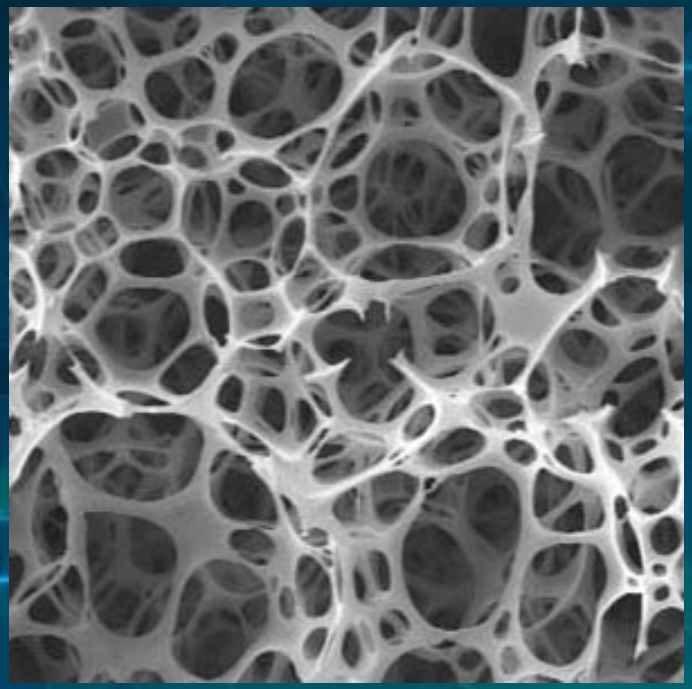


Porzioni di tessuto Adiposo possono essere ricostruiti dalla combinazione di cellule adipogeniche con scaffold 3D bioassorbibili in possesso di adeguate proprietà meccaniche



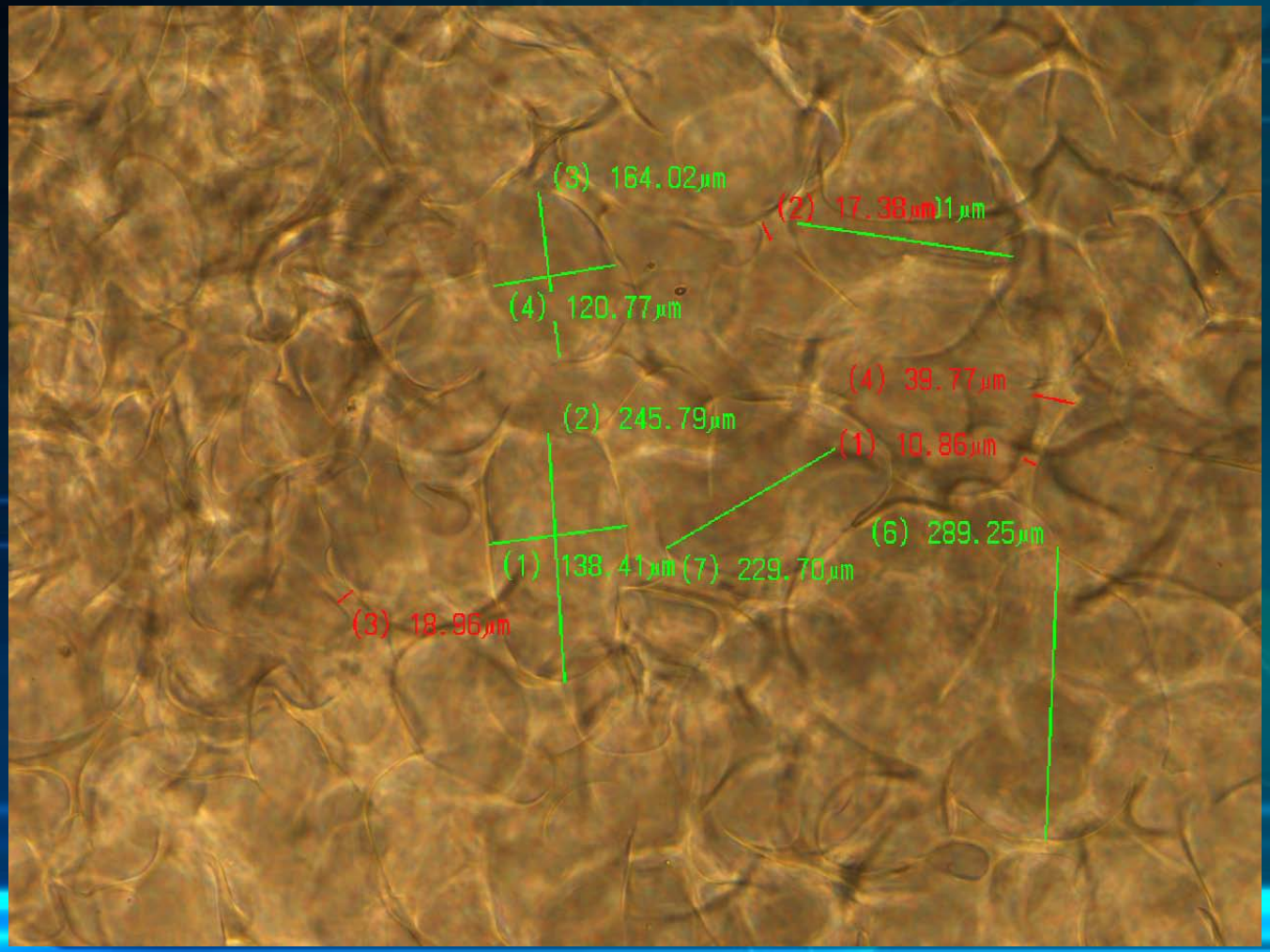
- 3d Scaffold
- Polimereri come “shape addresser”
-

I polimeri come "shape addresser"



Acido ialuronico - polimero

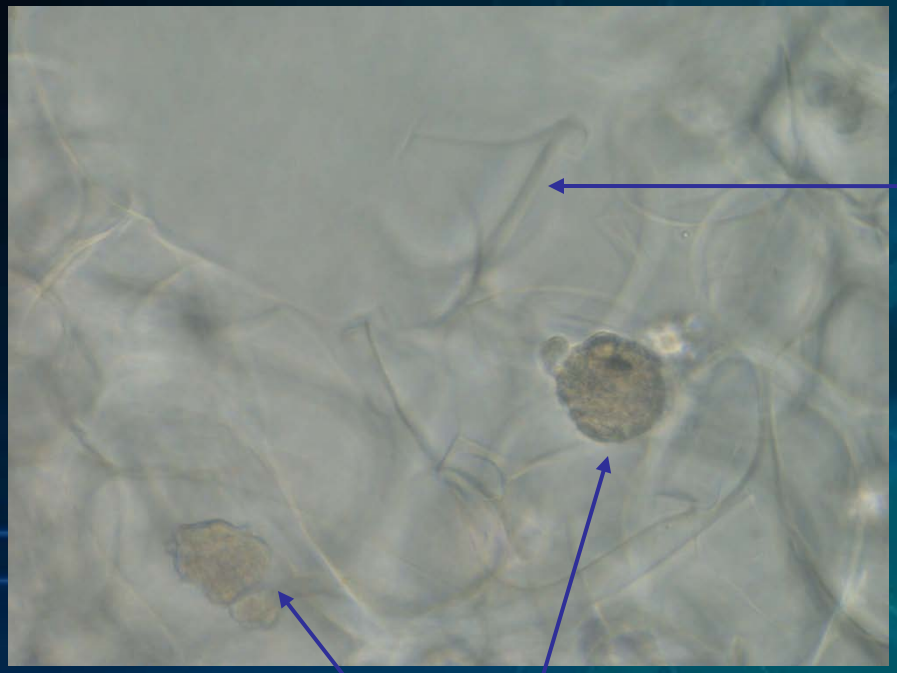
I polimeri come "shape addresser"



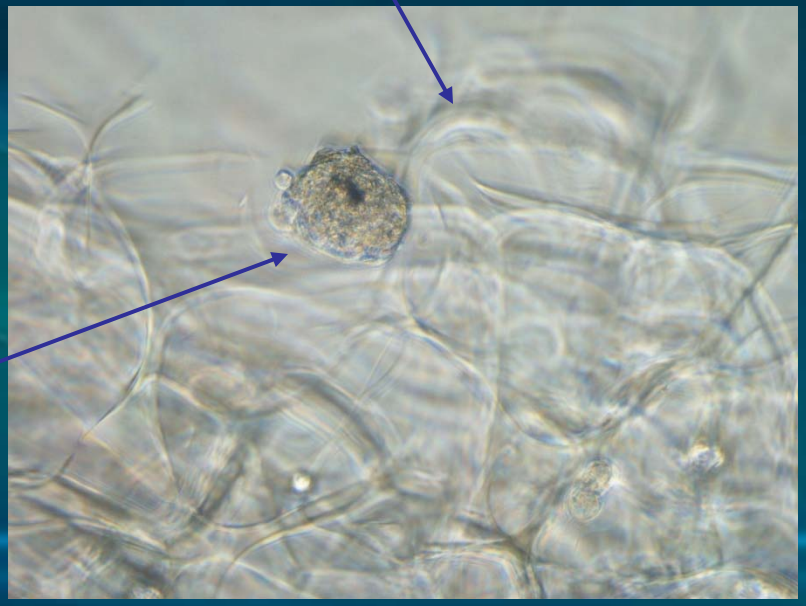
Acido Ialuronico HA6%

Hyaluronic acid-based scaffolds

In vitro probe



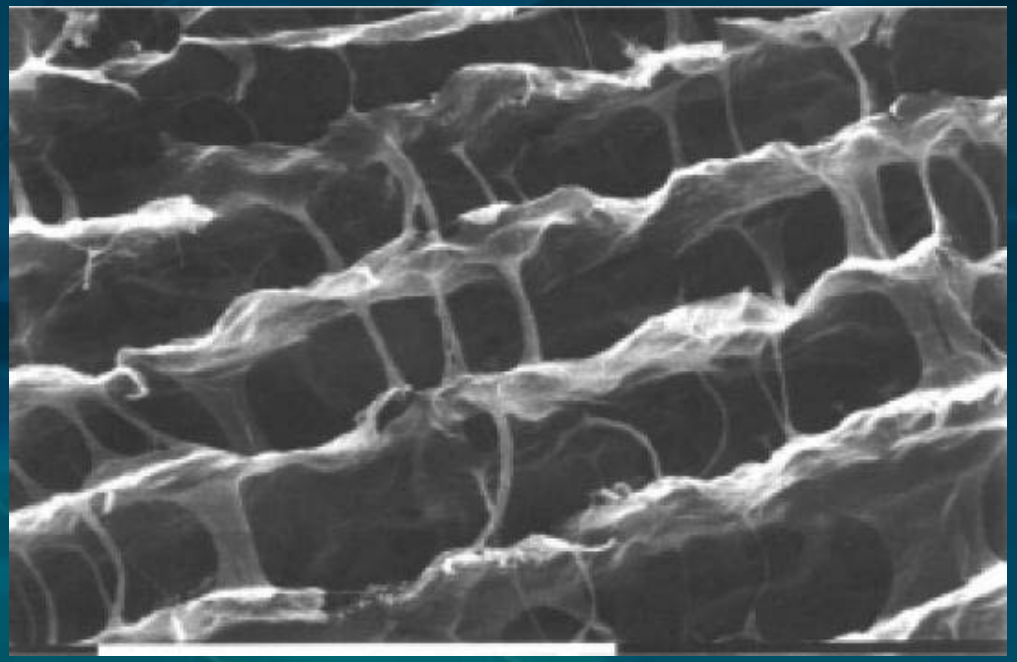
ACIDO IALURONICO



Cells

Collagen-based scaffolds

3D Collagen sponge

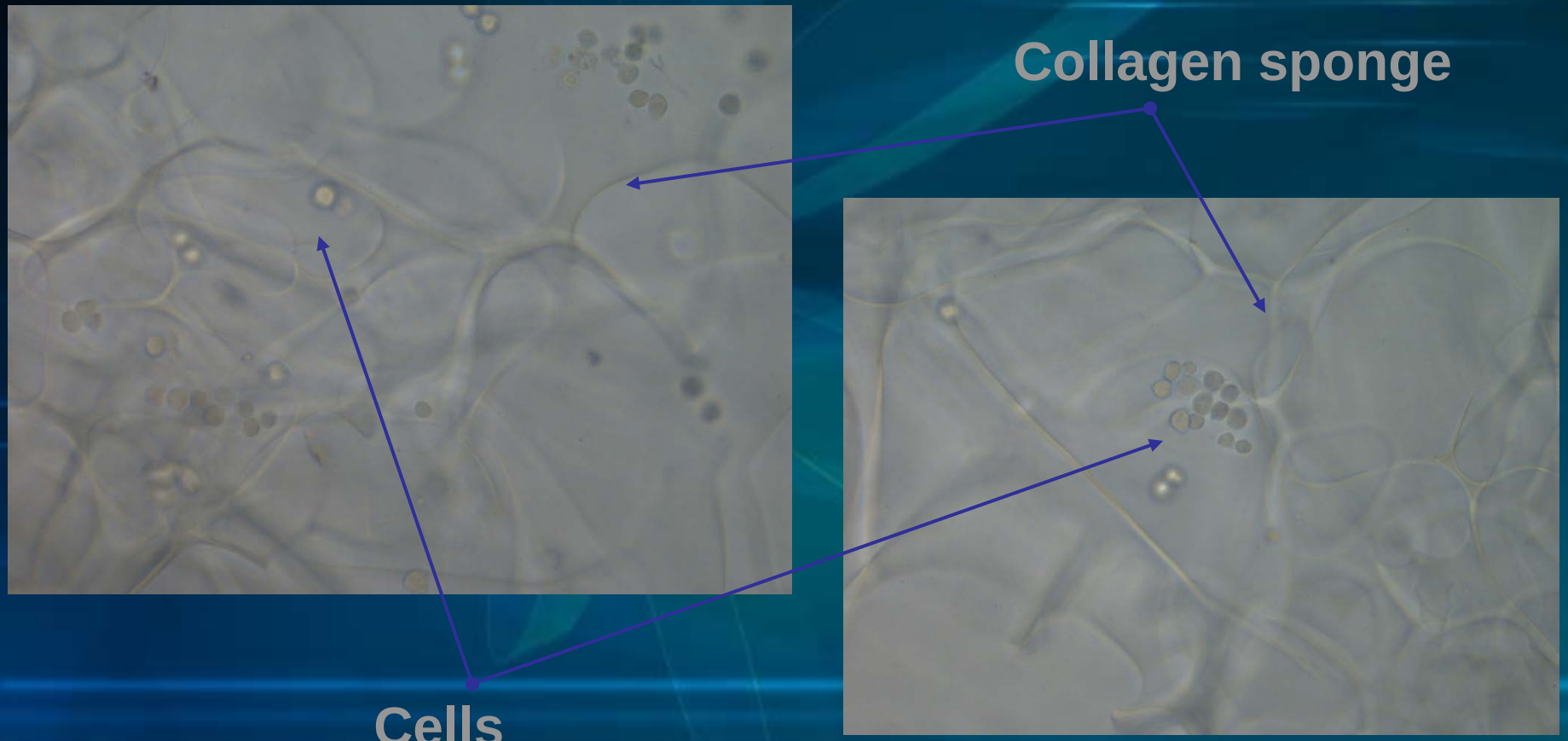


Collagen scaffold produced by directional solidification and subsequent vacuum-drying, showing a regular structure. Scale bar is equivalent = 0,1 mm

D. Von Heimburg et al. – Biomaterials 22 (2001) 429-438

Collagen-based scaffolds

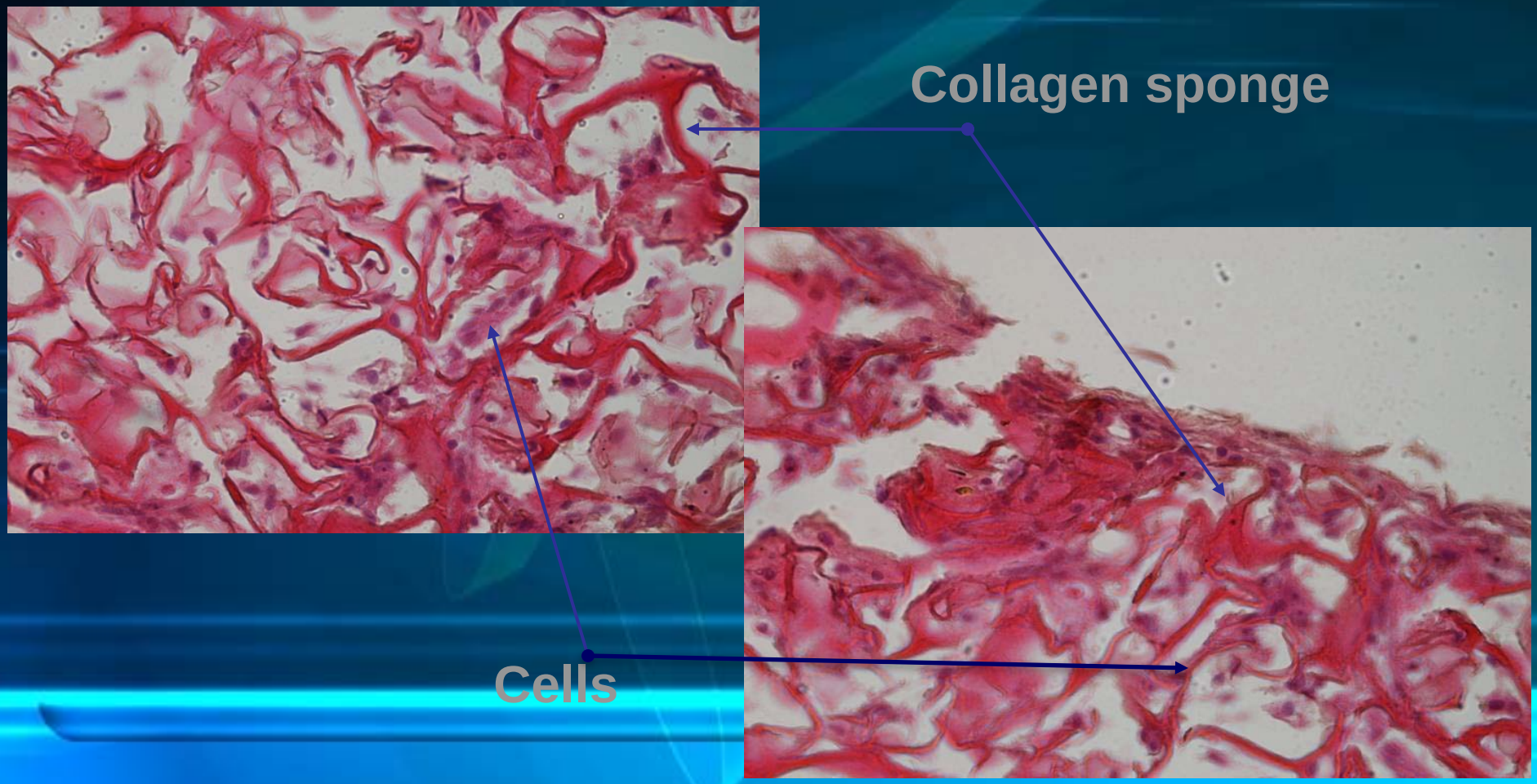
In vitro probe



Collagen-based scaffolds

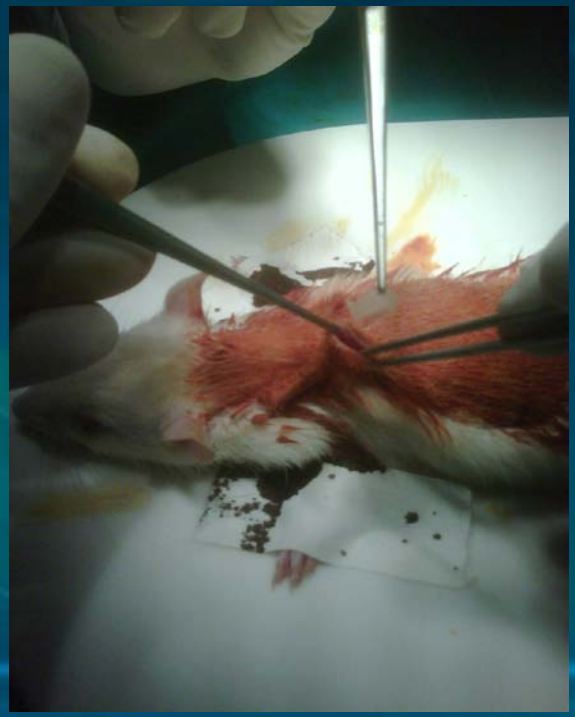
In vitro probe

Histologic staining



Fase 4

- Applicazione “*in vivo*” su ratti immunosoppressi

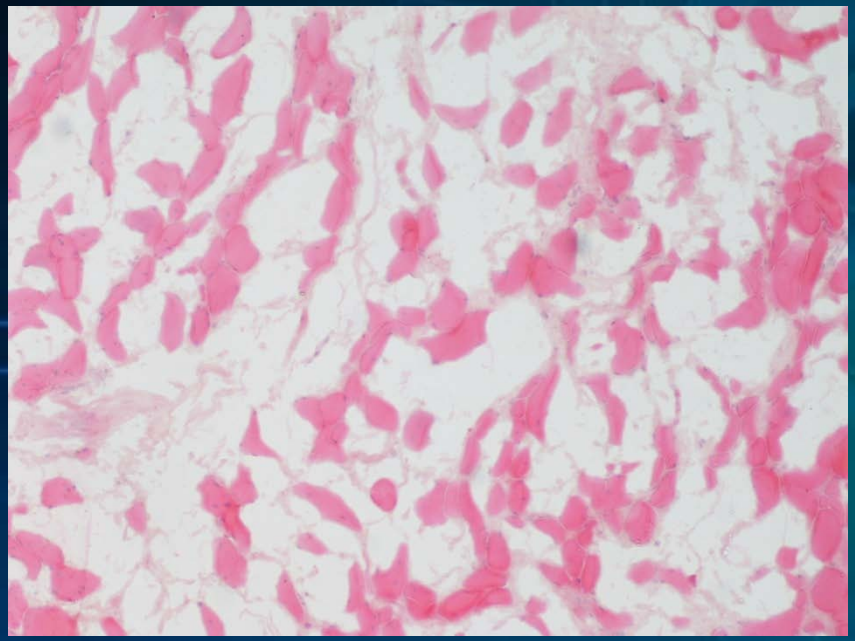


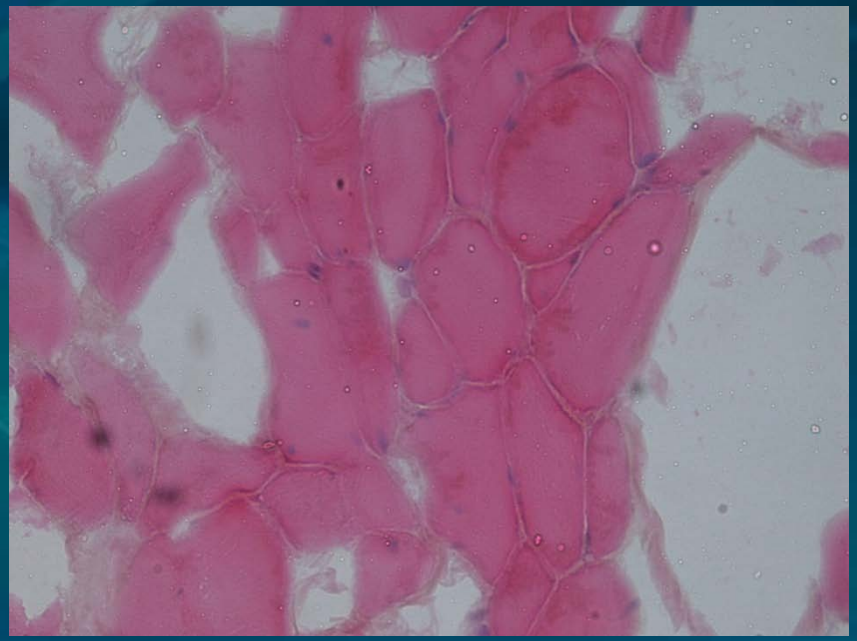
In Vivo

Acido Hialuronico + hASCs

In Vivo

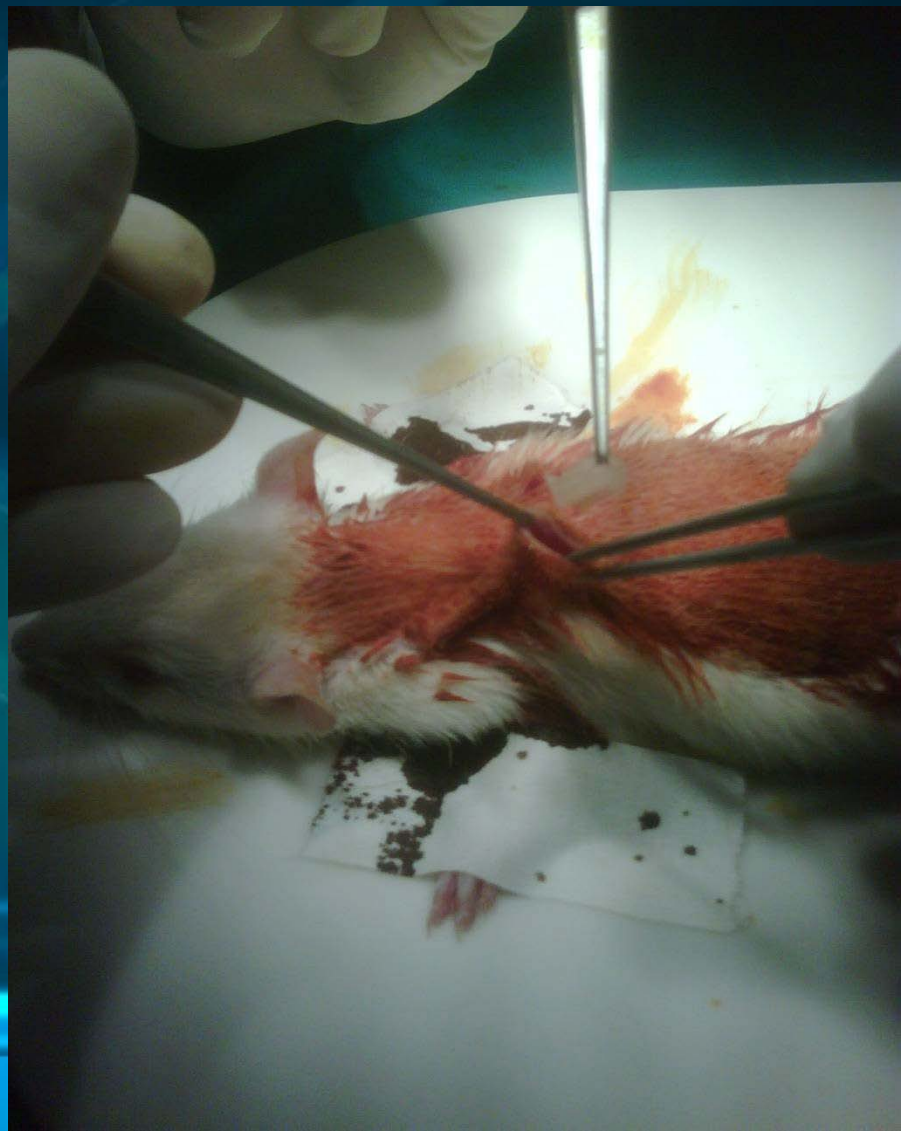
Acido Hialuronico + hASCs



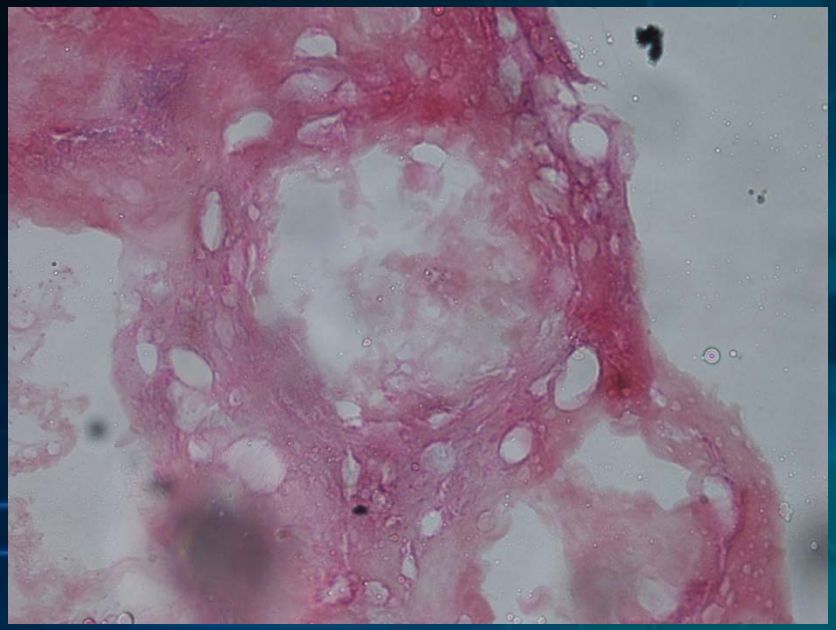


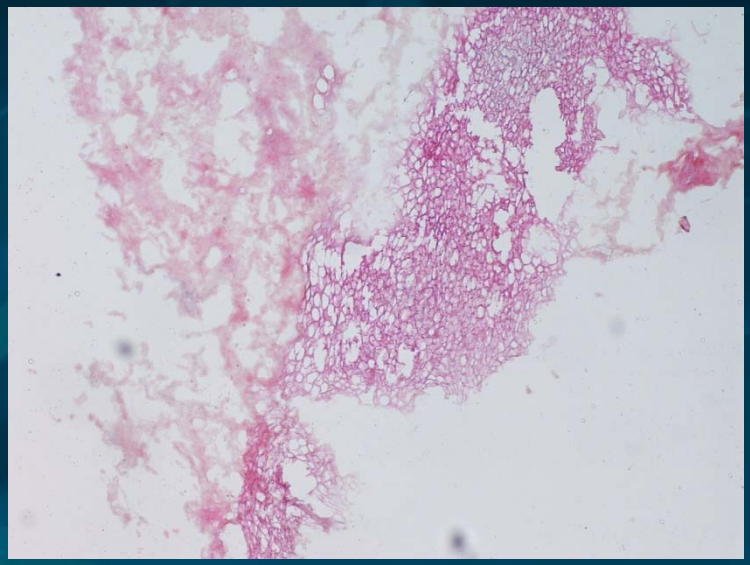
In Vivo – Work in progress

Spugnetta di Collagene e hASCs

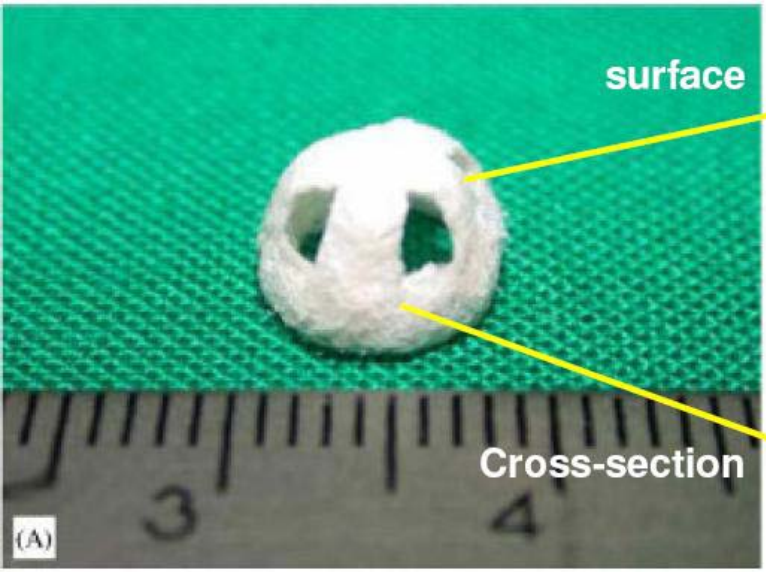


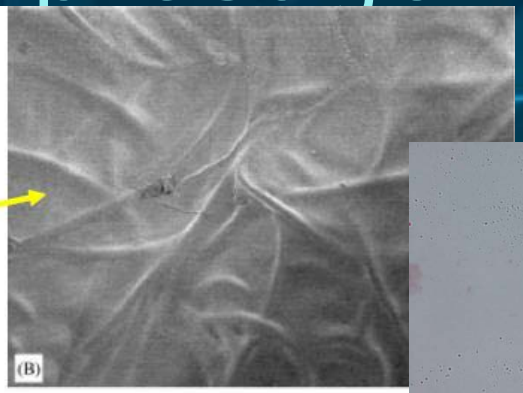
In Vivo

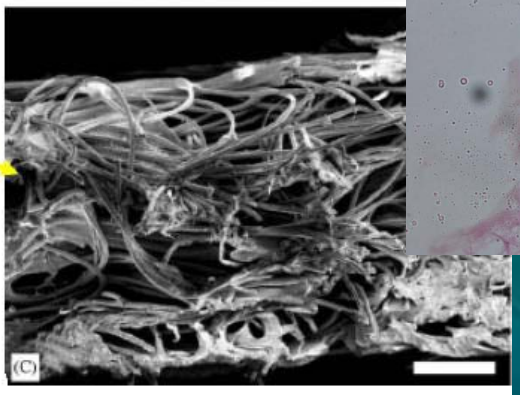


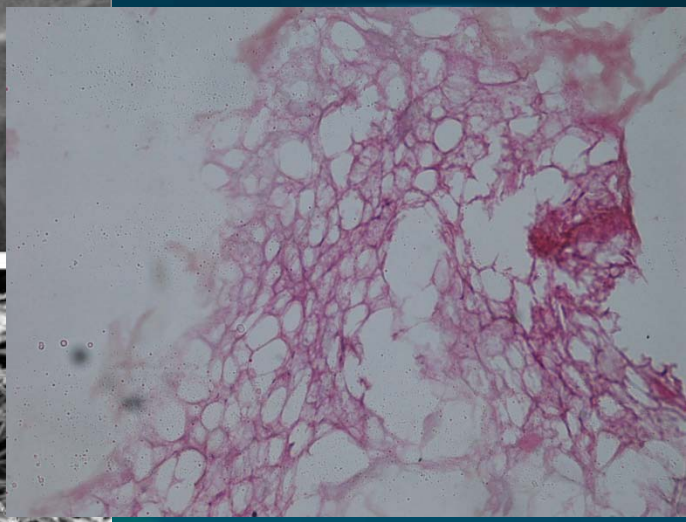


Fase 5
Work in progress……
scaffold presagomati









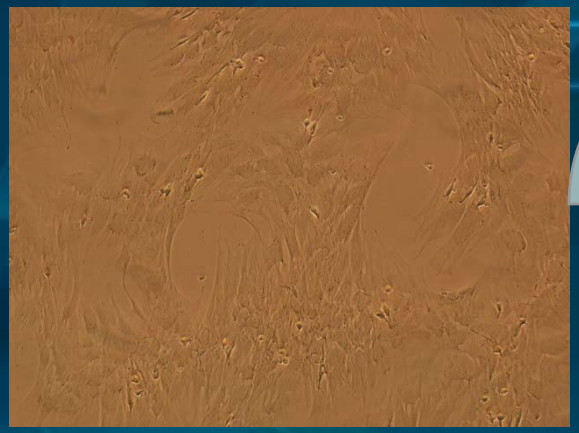
The Challenge

"I understand they're going to connect them. The Provost ordered it."

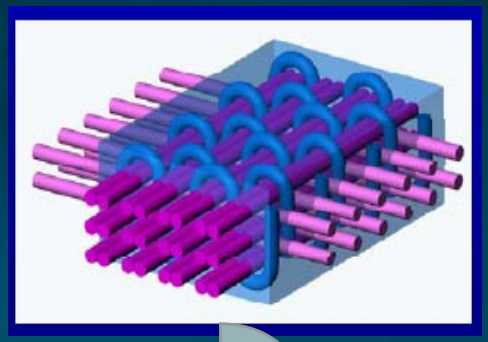
Tissue Engineering

Liposuzione per acquisire le cellule staminali autologhe

Isolamento

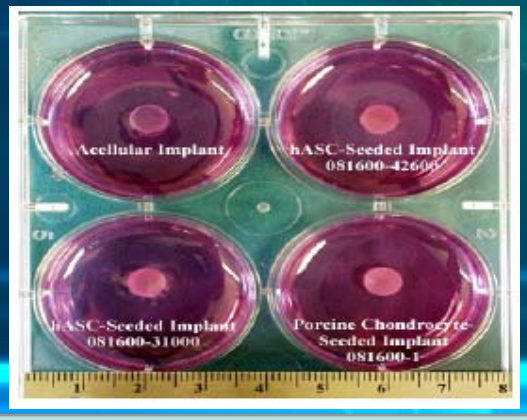


Preparazione dello scaffold 3D



Impianto delle cellule in uno scaffold 3D

Cultura in bioreattori (?)

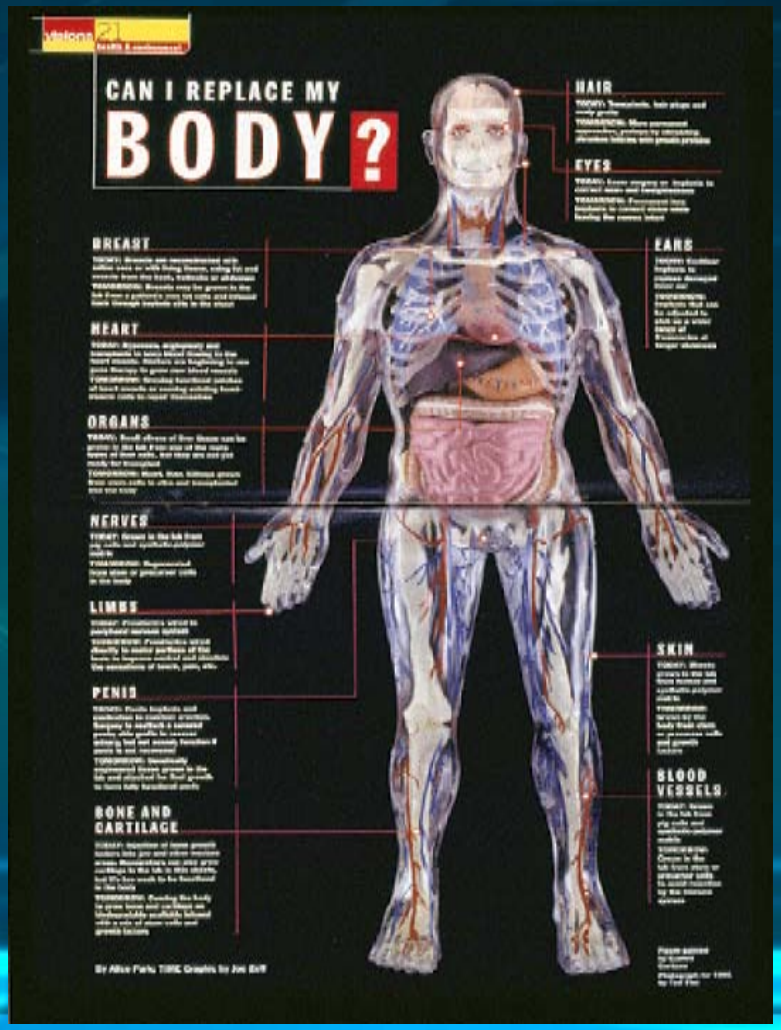


Implant

Ingegneria Tissutale:
semplificando il paradigma

- Cellule
- Biomateriale
- Fattori di crescita

Tessuti ed Organi



Conclusioni

- Il tessuto adiposo è una fonte abbondante di cellule staminali mesenchimali
- L’uso di mezzi specifici e scaffold 3D biocompatibili è possibile riprodurre in toto il tessuto adiposo in vitro e su modelli animali
- Il tessuto adiposo è disponibile per l’ingegneria dei tessuti e per la medicina rigenerativa.

- Grazie per la cortese attenzione!