Tissue Engineering Basic Concepts

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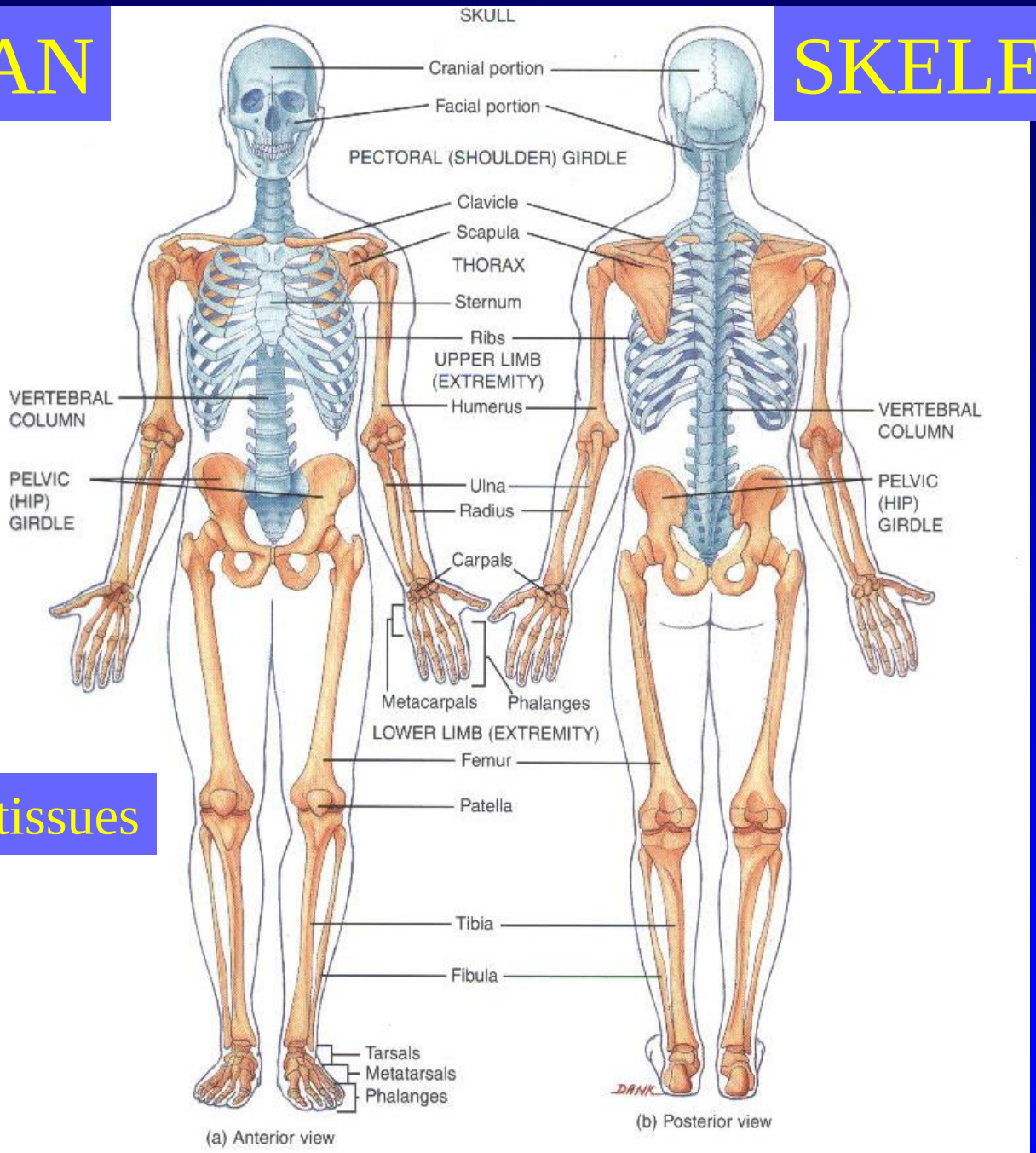
Tissue Engineering is involved with using cells and materials to form functional tissues or organs products

HUMAN SKELETON



Connective tissues

Tissue engineering

bone

tendon

nerve

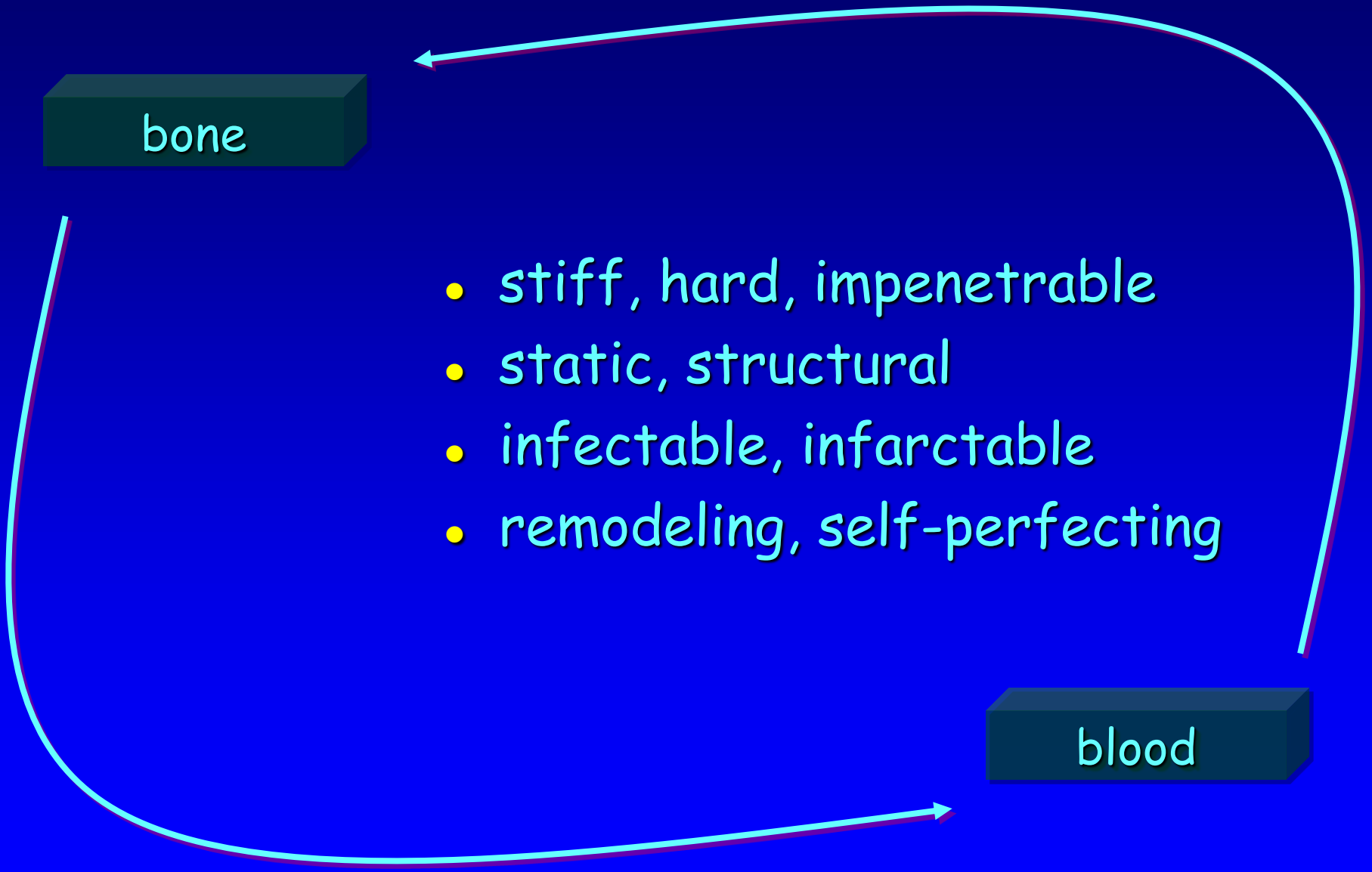
skin

muscle

liver

ligament

cartilage



- stiff, hard, impenetrable
- static, structural
- infectable, infarctable
- remodeling, self-perfecting

Principal

- all biomedical and bioengineering research and products must be driven by real clinical problems

bone

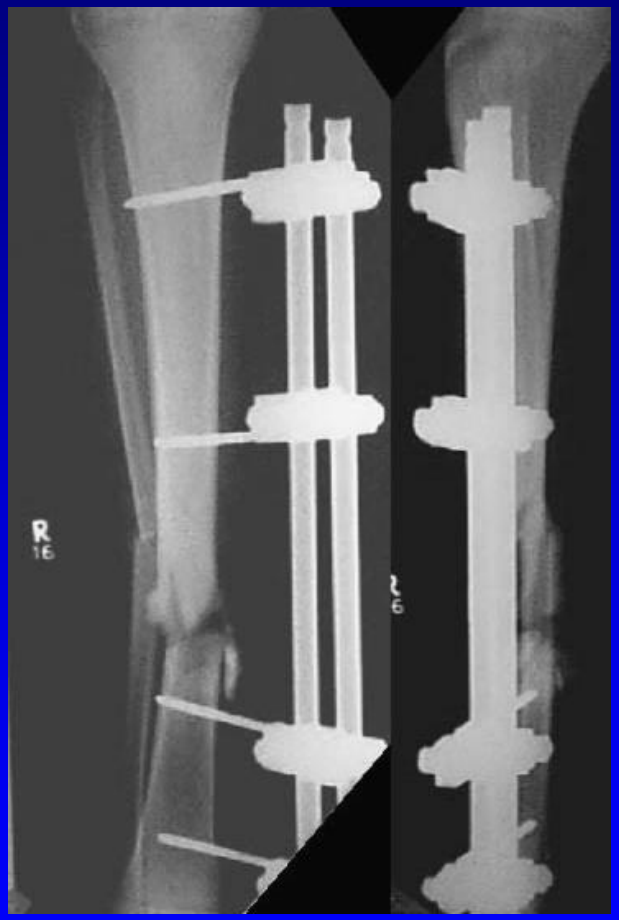
problems → models

- the high-energy fracture that may not heal
- the loose prosthesis with osteolysis
- the hard-walled osteomyelitis cavity

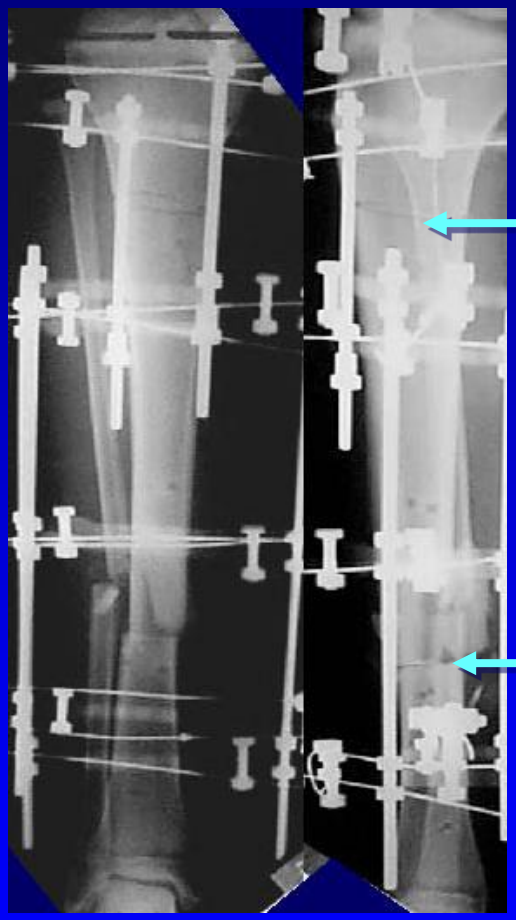
and

- the osteoporotic skeleton

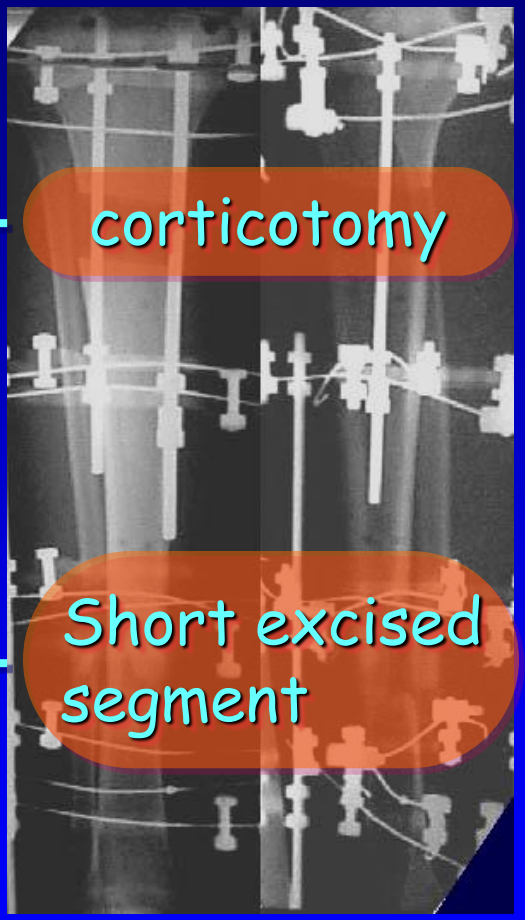
Sick whole tibia



8 weeks post-injury

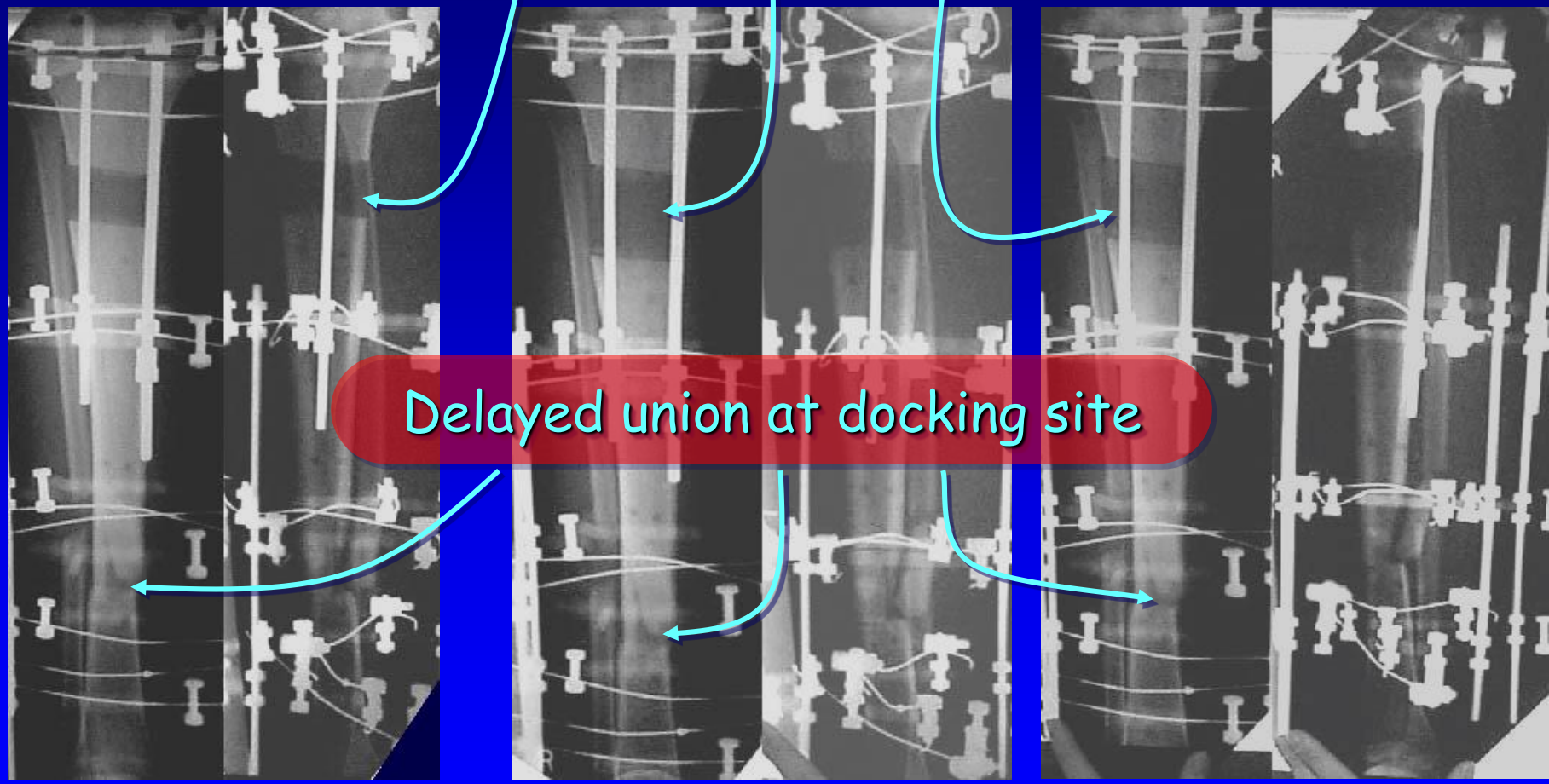


2 days later



2 months after frame

Stalled distraction osteogenesis



Delayed union at docking site

2 months

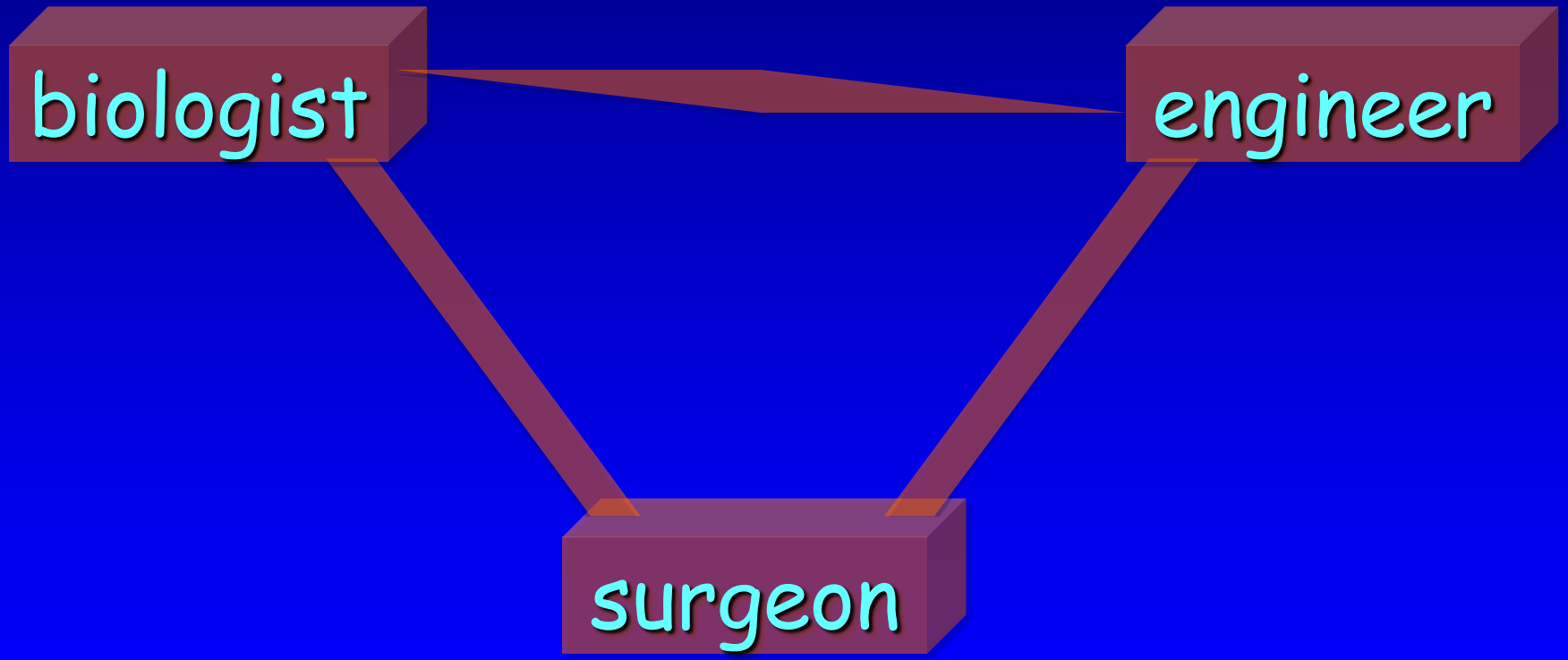
3 months

4 months

Tissue engineering

- administration of growth factors alone
- implantation of a synthetic device with or without growth factors
- implantation of cells alone
- implantation of cells and a synthetic device
  - 3-D culture

Tissue engineering



High energy tibial shaft fracture - the surgeon's dream

- On the night of injury:
  - ↗ excise non-viable tissue
  - ↗ stabilise
  - ↗ harvest bone marrow

- Following 2 weeks:
  - ↗ heal soft tissues
  - ↗ culture bone cells

- 3 weeks after injury
  - ↗ deliver bone cells to fracture site by local injection

High energy tibial shaft fracture - the engineer's dream

- Design a ceramic implant that replaces the damaged segment
- Watch the body populate it with cells and bring it to life
- Get paid more than the surgeon

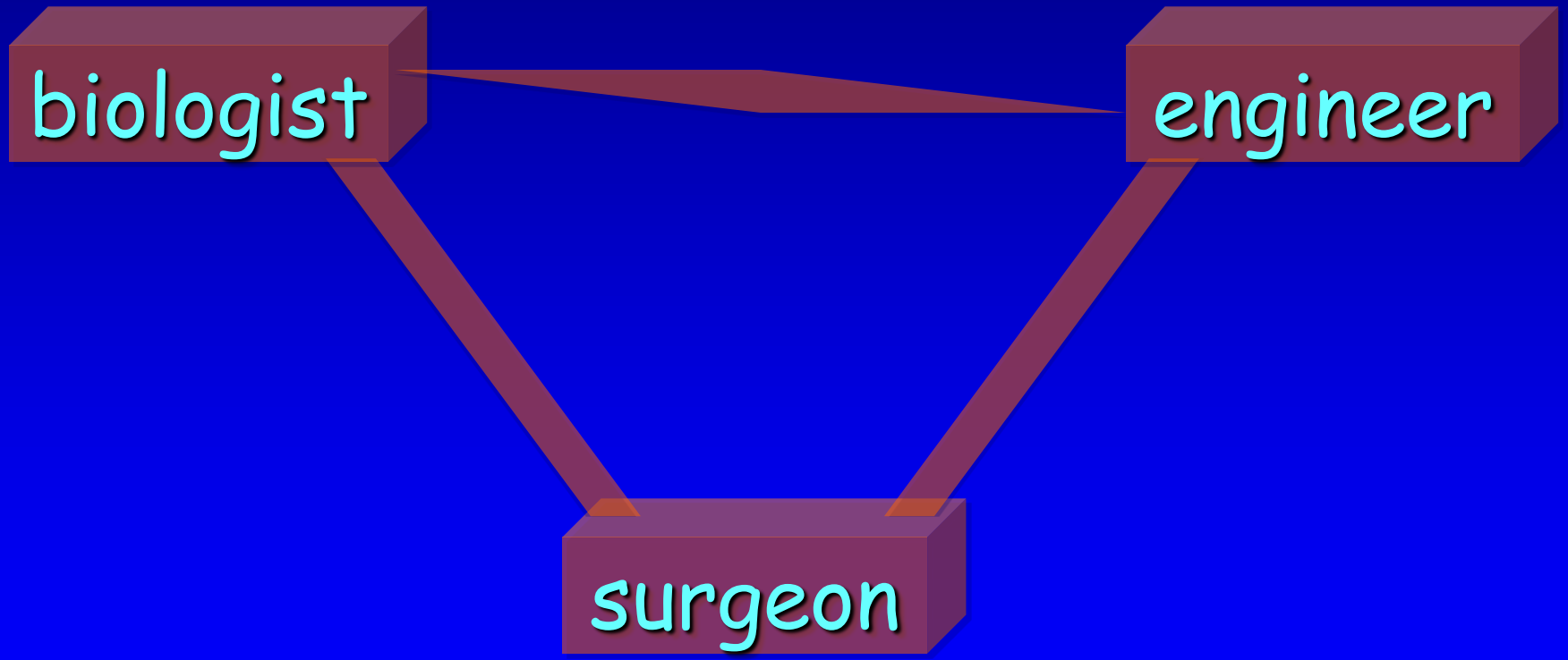
High energy tibial shaft fracture - the biologist's dream

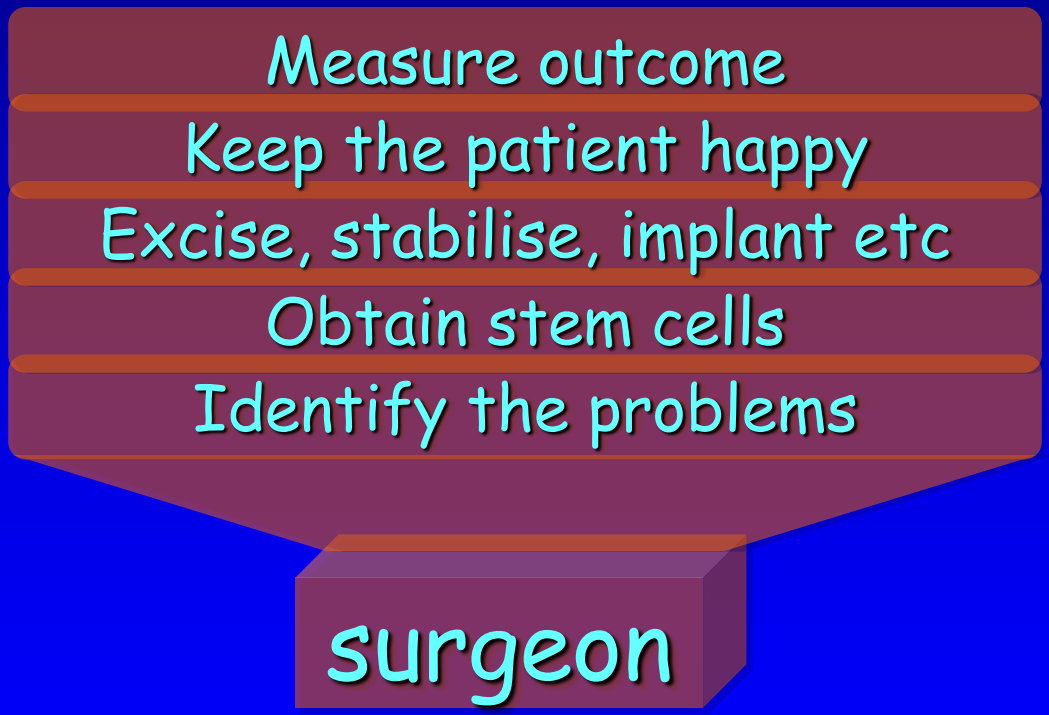
- Take a blood sample, extract stem cells from it, culture them

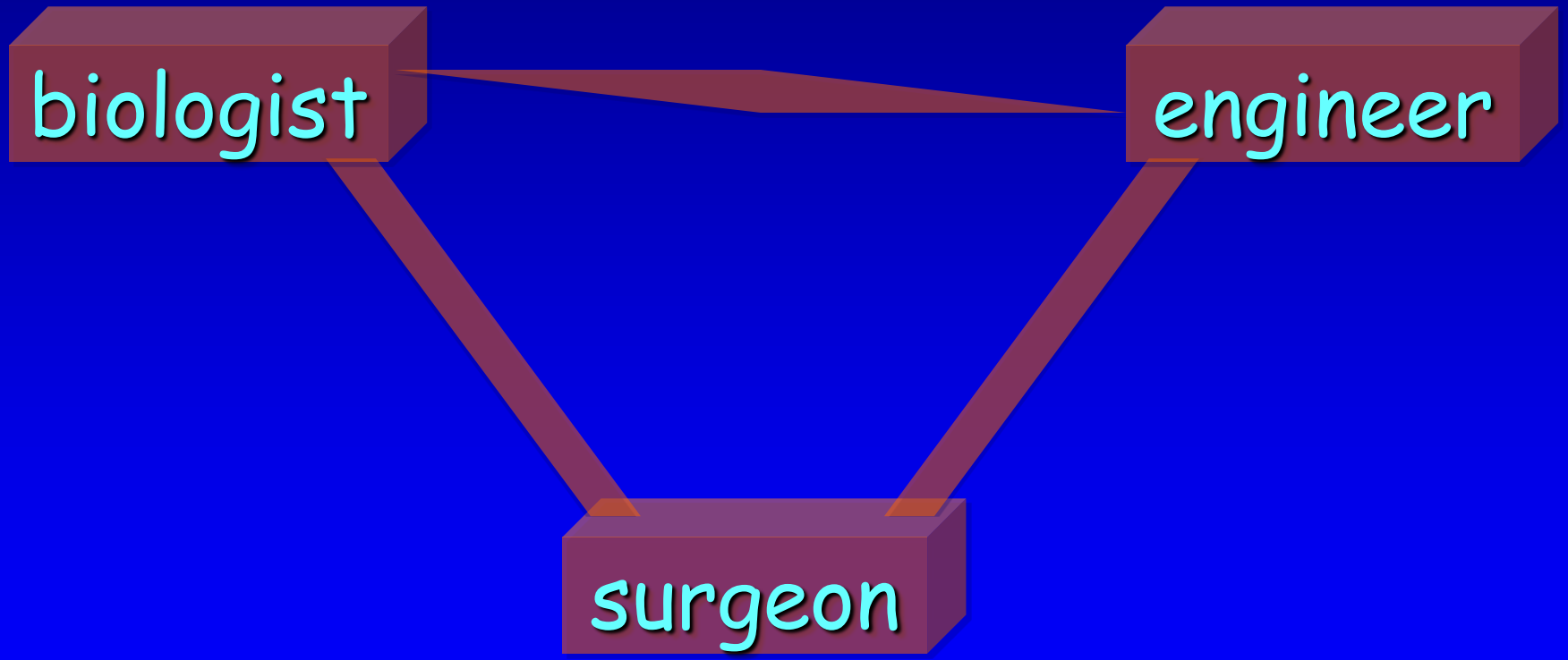
- Transfect them with genes to home in on the damaged tissue, induce angiogenesis and bone formation. Inject them IV.

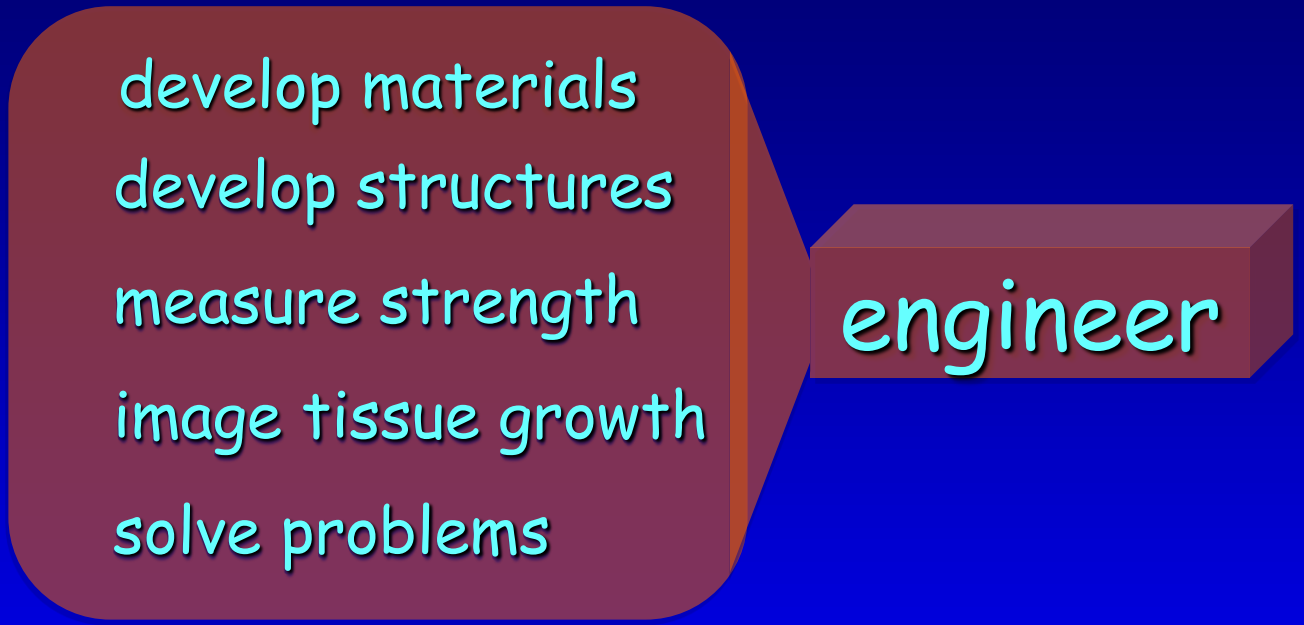
- Sack the surgeon

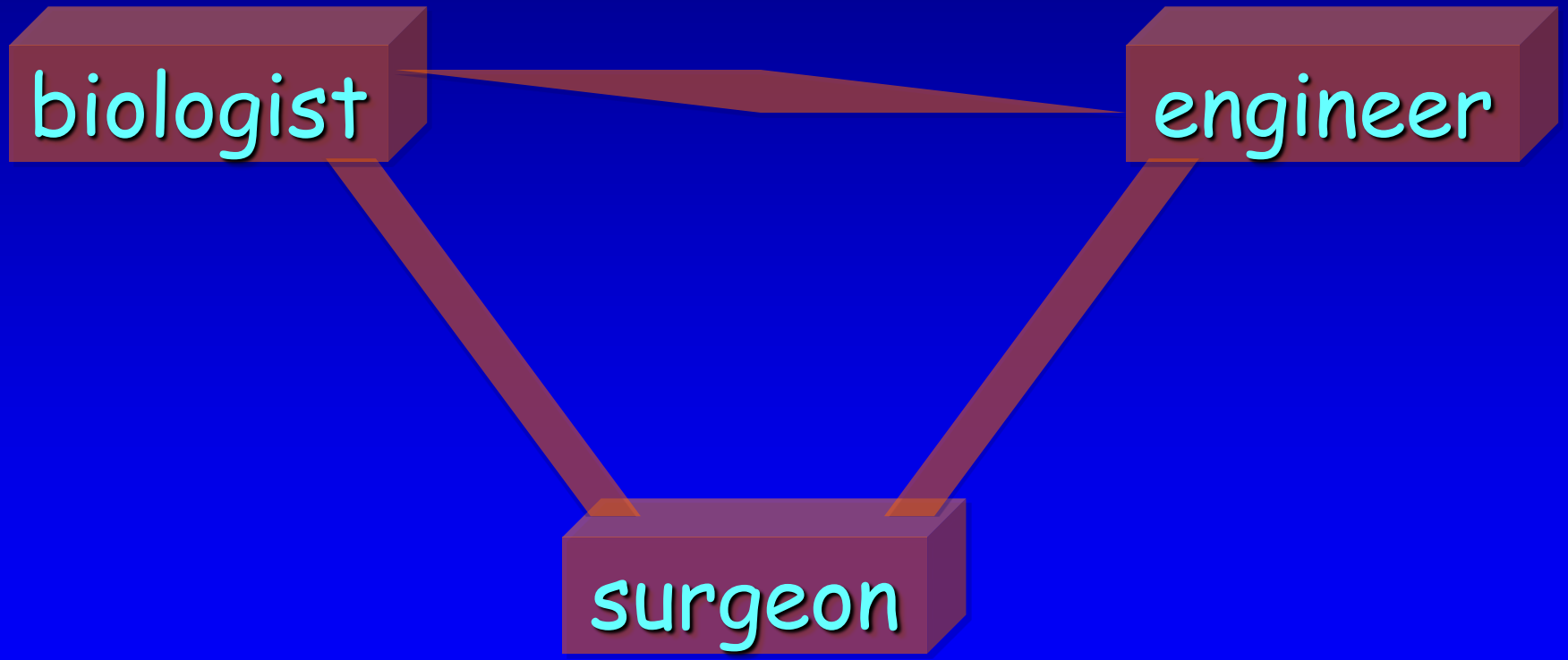
Tissue engineering

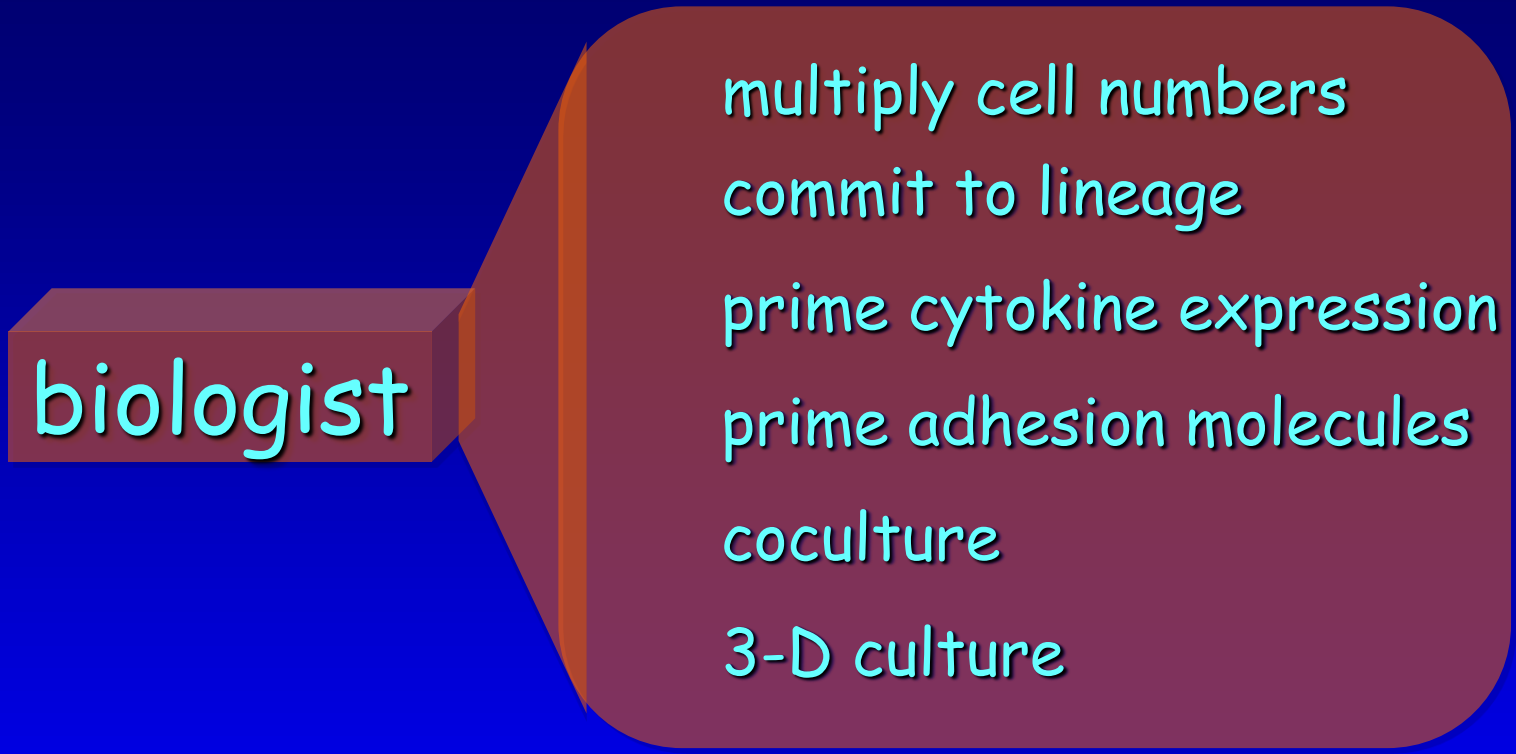



Measure outcome
Keep the patient happy
Excise, stabilise, implant etc
Obtain stem cells
Identify the problems
surgeon


biologist
engineer
surgeon


develop materials
develop structures
measure strength
image tissue growth
solve problems
engineer


biologist
engineer
surgeon


biologist
multiply cell numbers
commit to lineage
prime cytokine expression
prime adhesion molecules
coculture
3-D culture

Key problems

- Cells or stem cells
- Scaffoldings
- Vascularisation

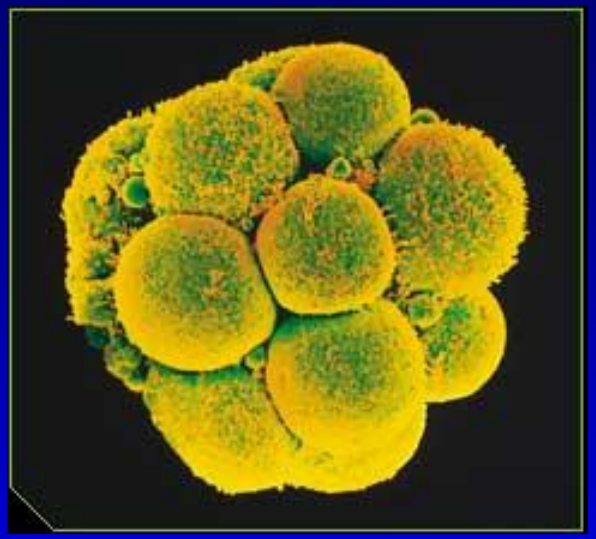
Tenets

- use natural routes
- design protocols not just implants

Stem cells

- harvesting
- proliferation
- *in vitro* conditioning
- delivery

What is stem cell ?



A cell with a capacity for **self-renewal** and the **potential to generate several types of differentiated progeny**.

On division, a stem cell can generate at least one new stem cell.

- Symmetric (producing two stem cells)
- Asymmetric (producing one stem cell and a daughter progenitor cell).

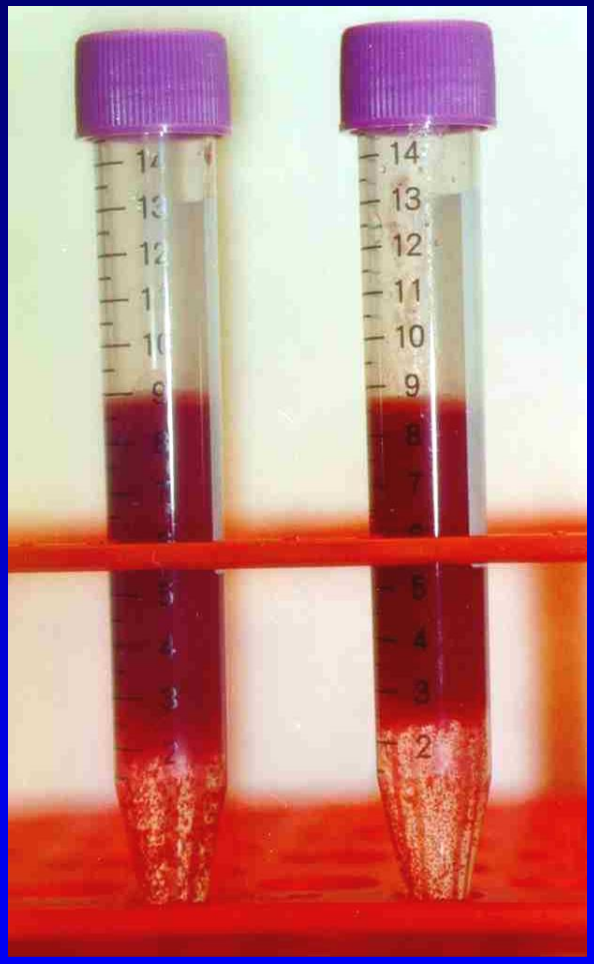
Progenitor cells generally divide faster than stem cells, but are more restricted in their developmental potential. **The stem cells are usually non-adherent to plastic wares.**

Sample Processing – Bone Marrow

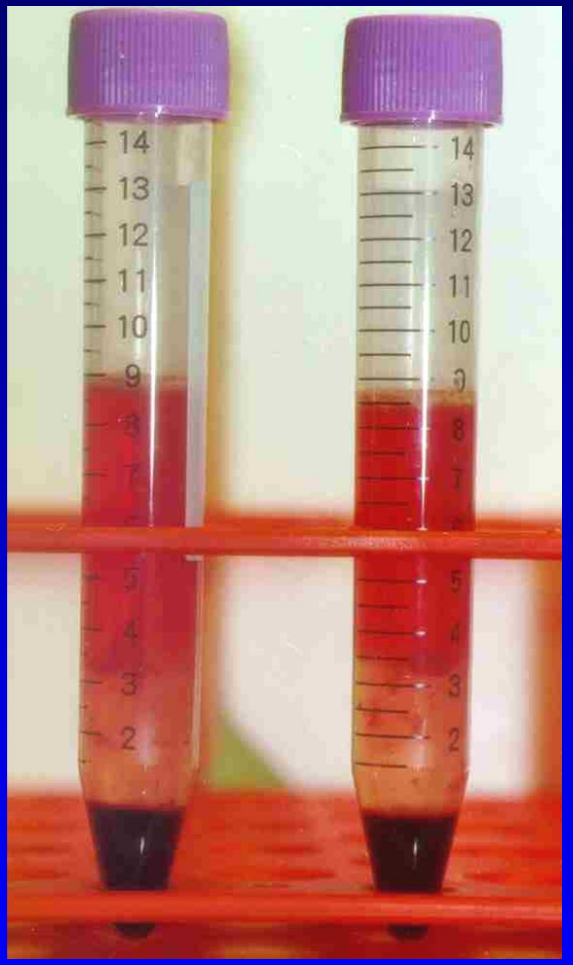
- ASPIRATED: green needle (8-10 times)
- DILUTED: PBS/TCM, to 12ml (1:2 approx)
- CENTRIFUGED: layered over lymphoprep (1:2), 800g, 30min, RmT°C

Human Bone Marrow - Aspirating

Sample Processing – Bone Marrow



Pre - Centrifugation

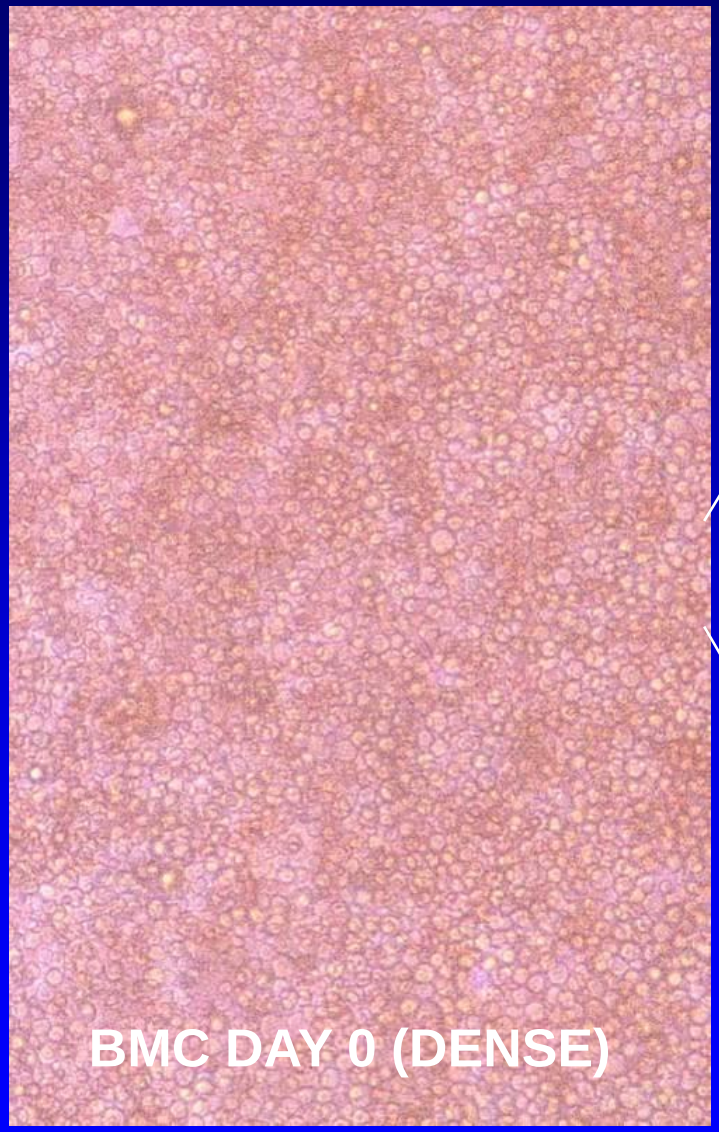


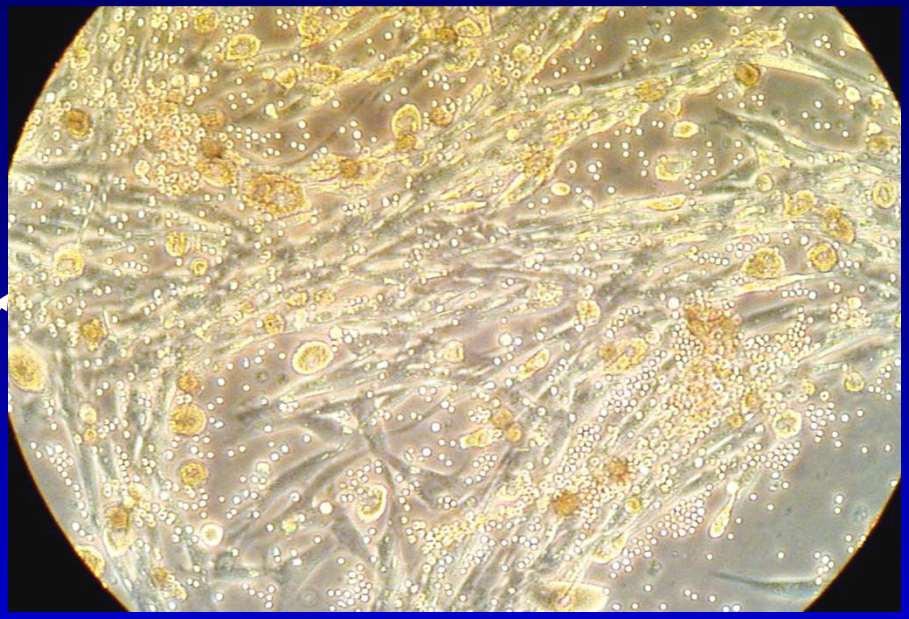
Post - Centrifugation

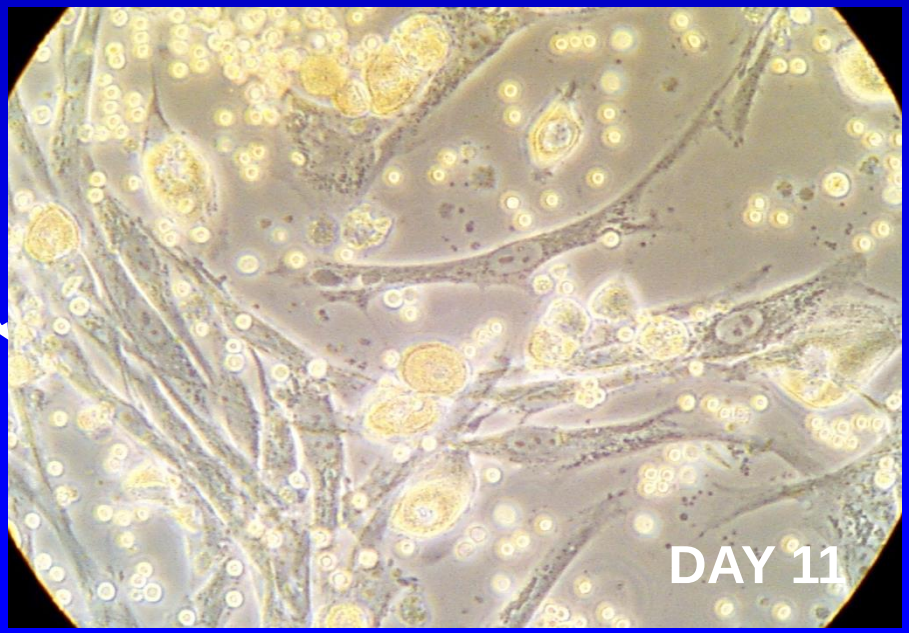
Cell manipulation

- culture conditions
  - ↗medium
  - ↗3-D culture
  - ↗coculture
- growth factor therapy
- gene transfer

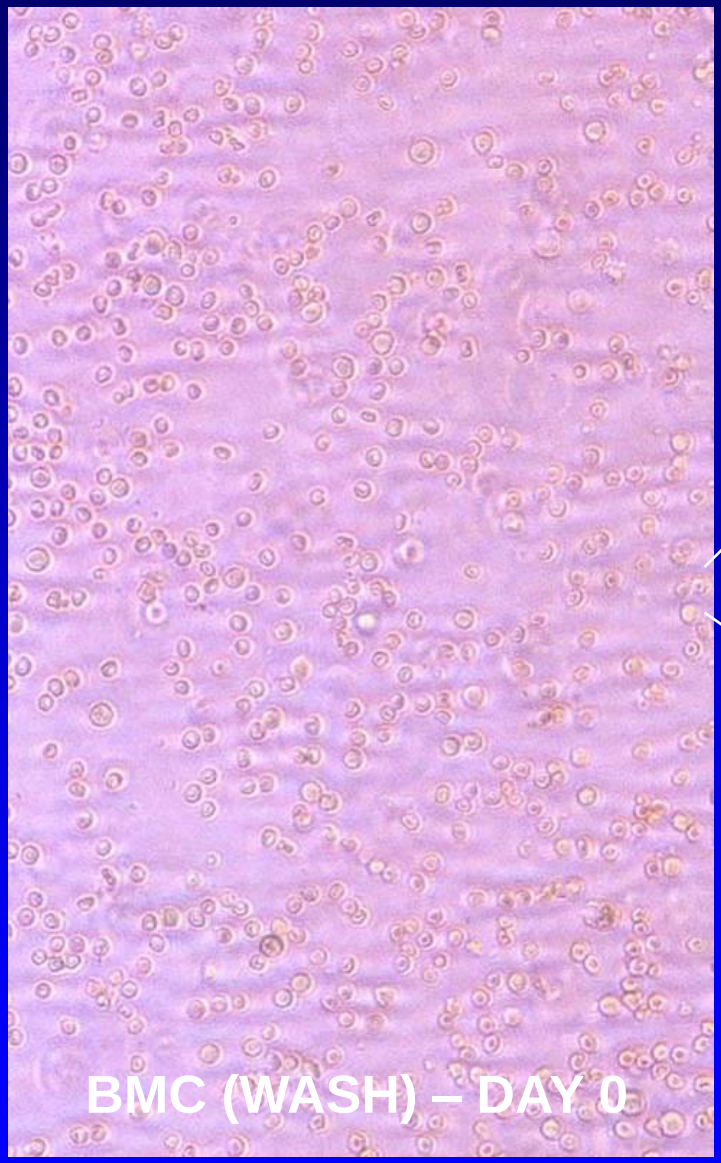
Sample Culture – Bone Marrow

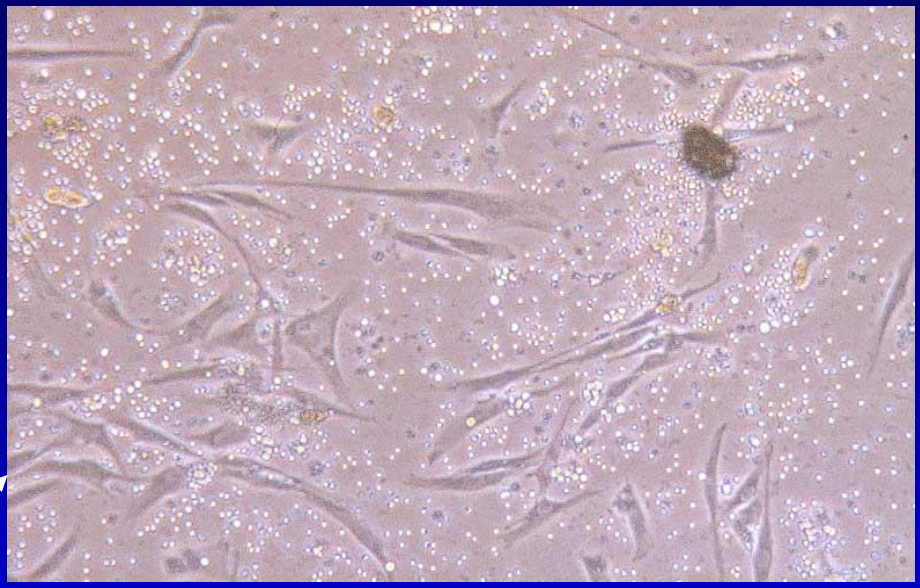


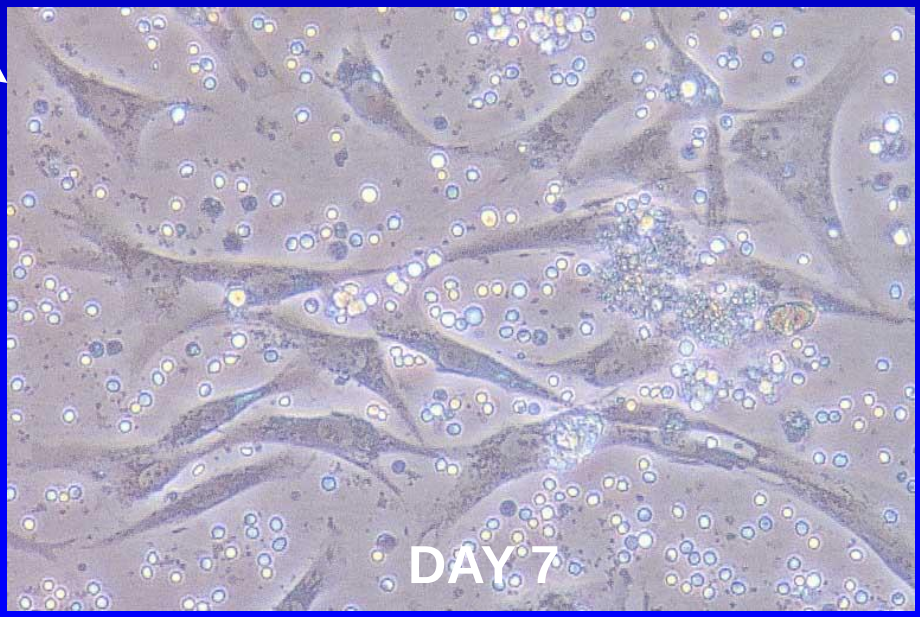




Sample Culture – Bone Marrow







The effect of growth factors on MSCs proliferation

	Dose 1 /ml	Dose 2 /ml	Dose 3 /ml	Dose 4 /ml	Dose 5 /ml
FGF-2	0.01ng	0.1ng	1ng	2.5ng	5ng
BMP-2	1ng	5ng	10ng	50ng	100ng
PTN	1pg	5pg	10pg	50pg	100pg
VEGF	1ng	5ng	10ng	50ng	100ng
IL-6	1ng	5ng	25ng	50ng	100ng
IL-6R	1ng	5ng	25ng	25ng	100ng
SCF	1ng	5ng	10ng	25ng	50ng

Doses are based on various previous publications.

Rotary Cell Culture System™

A bioreactor

A superior in vitro environment for studying: effects of intercellular communication, differentiation and function, phenotypic instability, and the inter and intracellular signaling pathways involved in clonal expansion.

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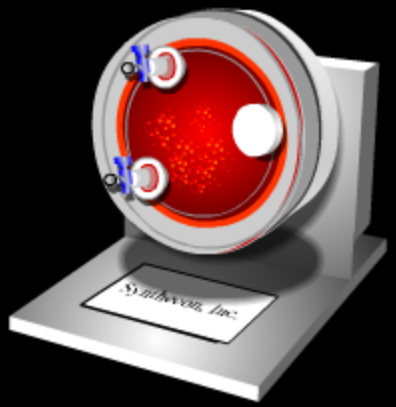
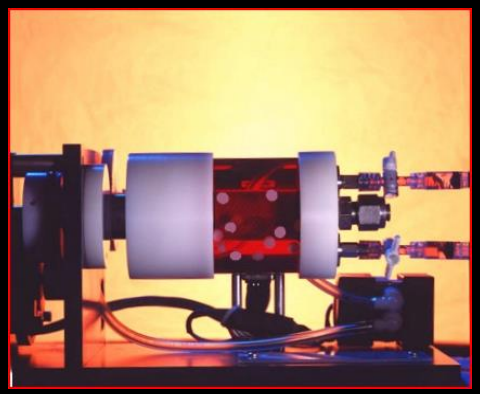
How It Works
Rotary Cell Culture System™

Since The Rotary Cell Culture System™ has no impellers, airlifts, bubbles, or agitators, tissue damage from impact and turbulence is significantly decreased as compared to conventional bioreactors. Shear stress and damage is so low that it is essentially insignificant.

The tissuse/cells would fall, tumble, mix in the media and without any single gravity vector dictating growth direction; the tissue/cells would grow in all directions. That is mechanism of the RCCS™.

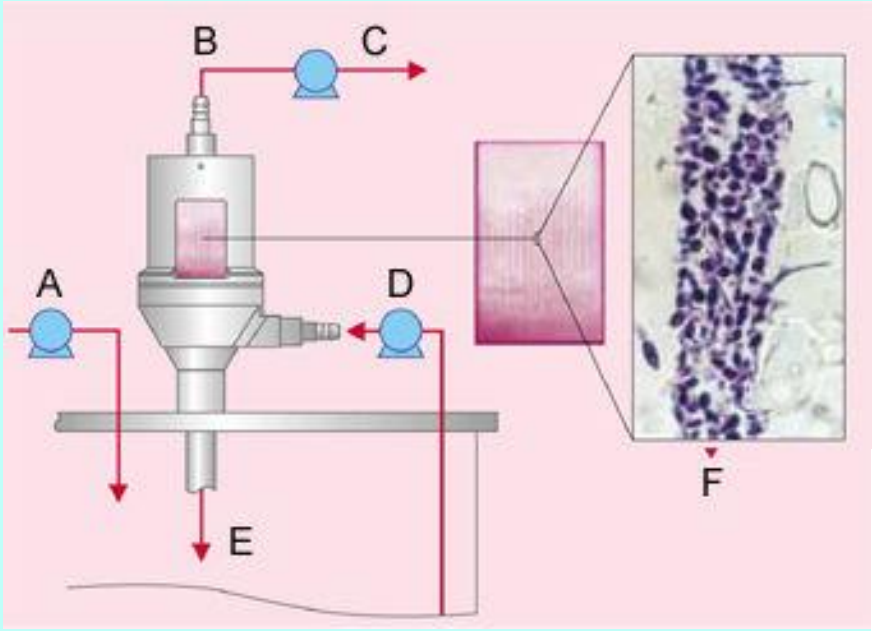


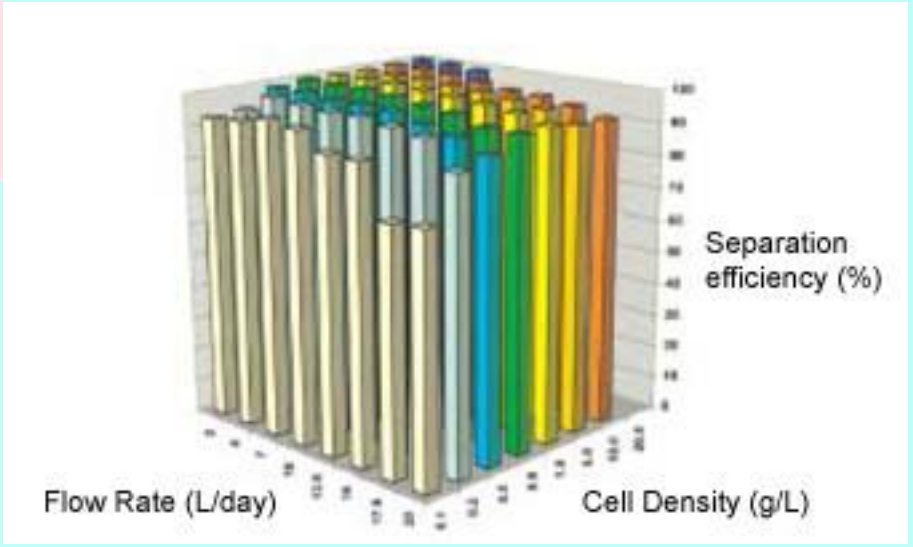


The operation of Synthecon's RCCS™ differs from all other cell culture systems. The cylindrical culture vessel is filled with culture fluid and the cells or tissue particles

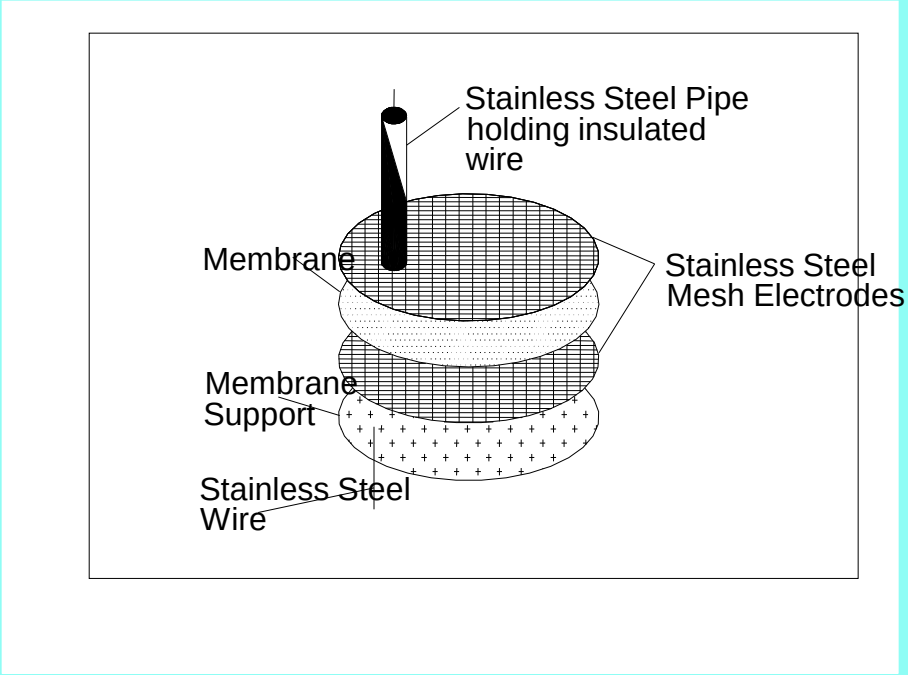
one Internet

Ultrasonic Perfusion Bioreactor





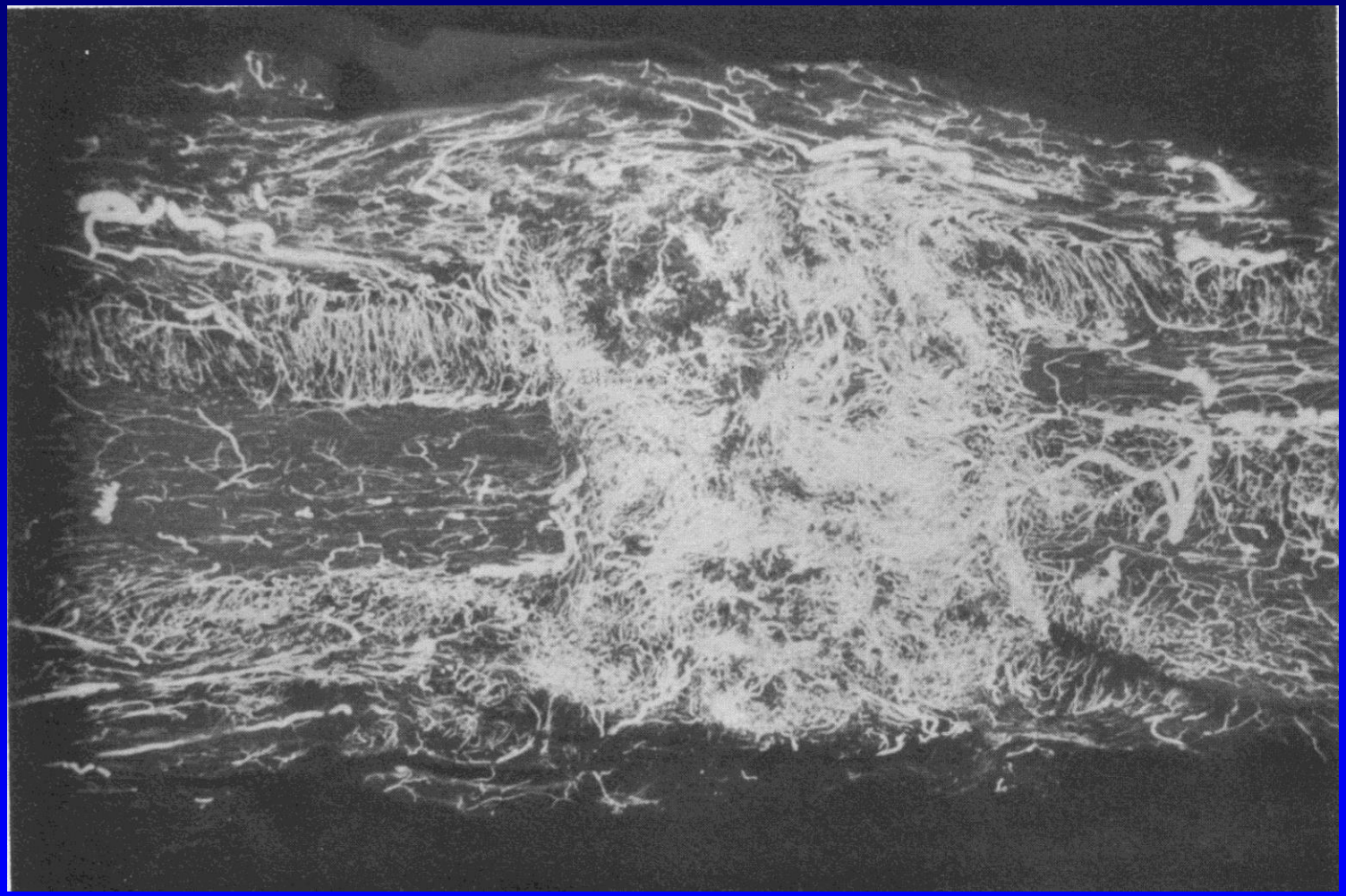
An integrated Membrane Bioreactor Equipped with Electro-osmotic Membrane Cleaning and Cell Suspension System



Vascularisation

- angio-induction
- angio-conduction
- implant cell survival

Microangiogram showing vascularity throughout autogenous cancellous graft / recipient bed



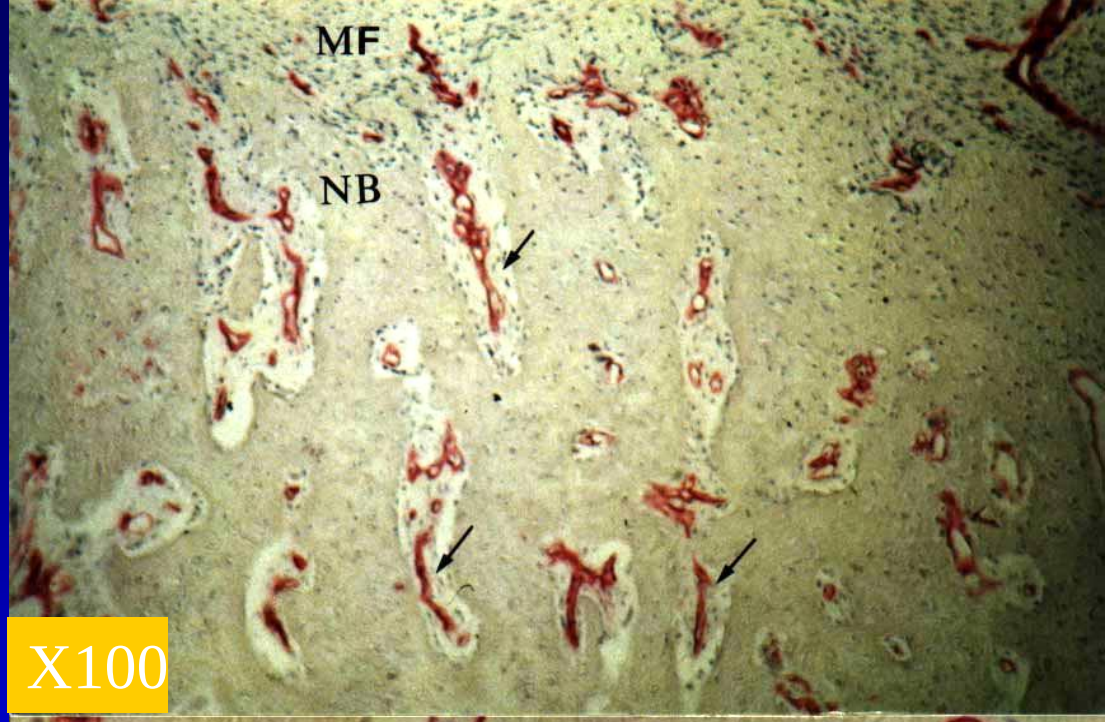
Angiogenesis Study

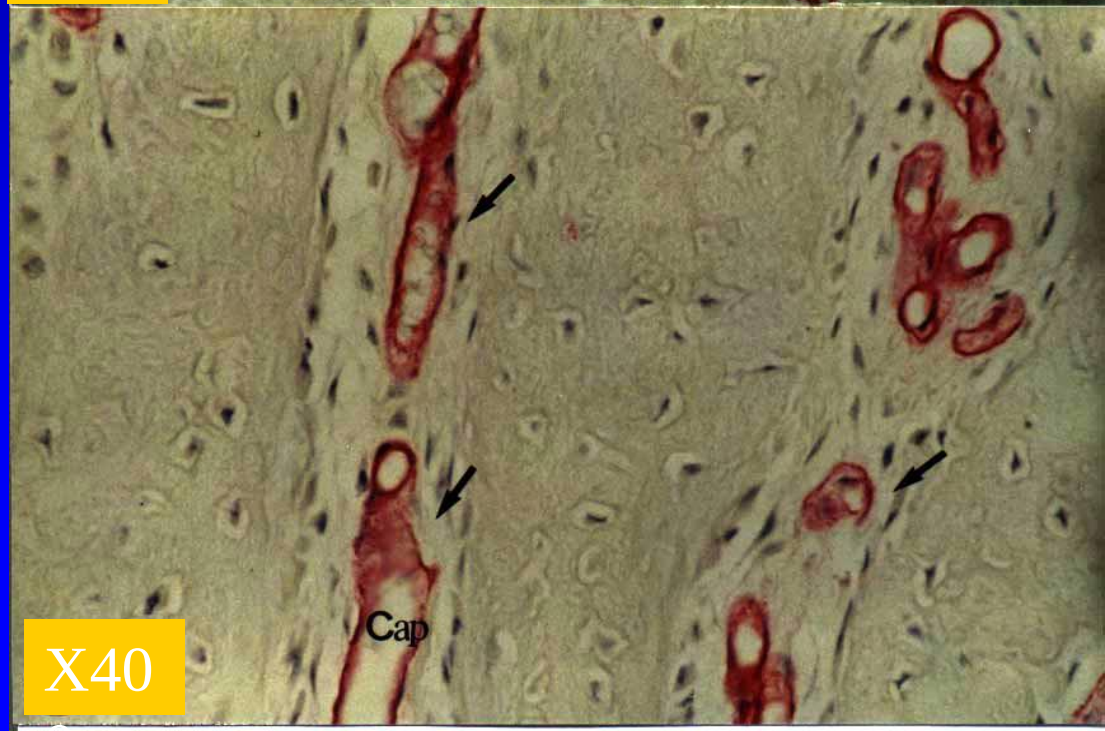
Immunostaining using MAb for type IV collagen

1.3 mm/day

New Bone Zone

Li, et al.
J Orthop Res
17:362-67, 1999





Animal models

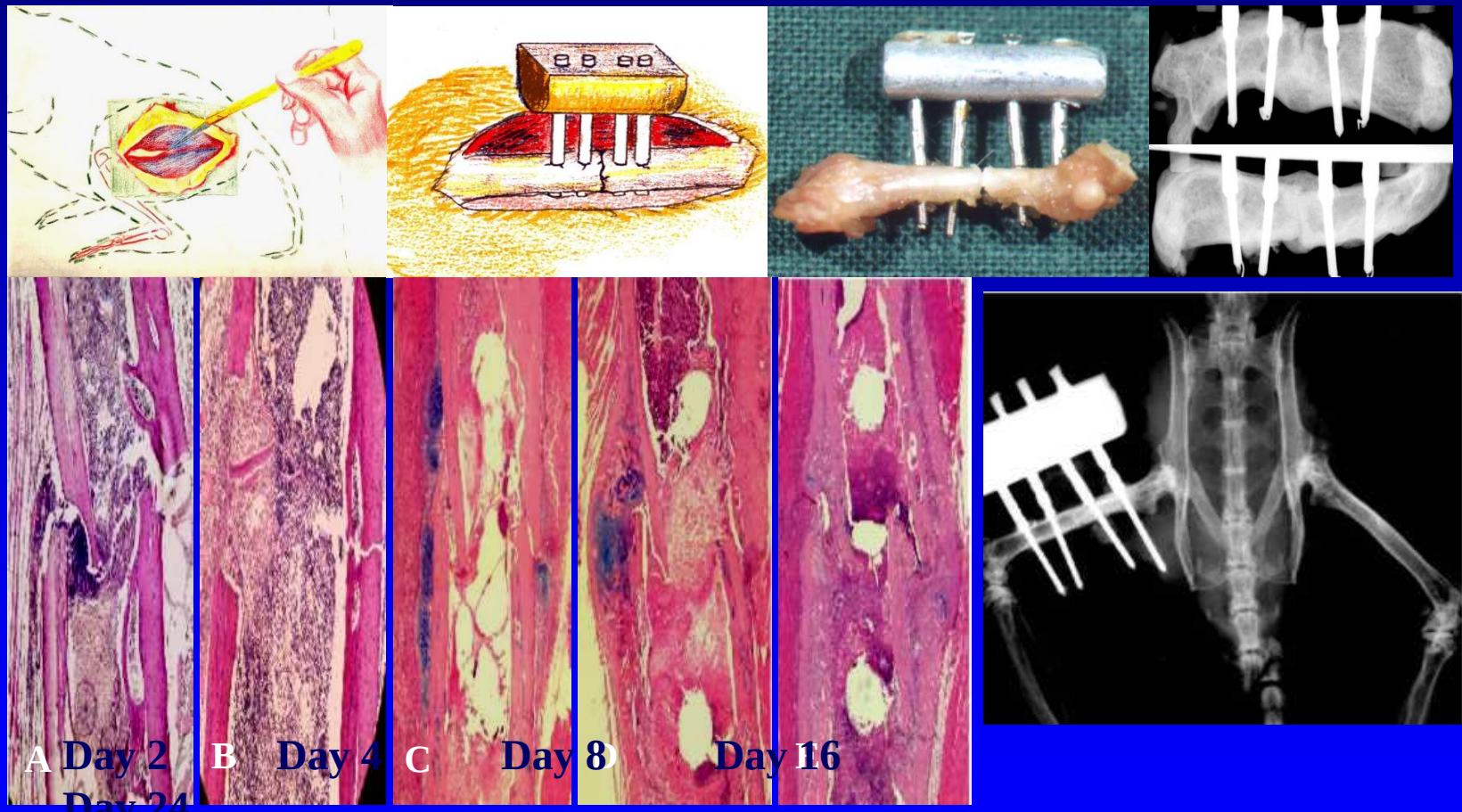
- mouse fracture
- Nude mice implantation model
- rabbit distraction osteogenesis
- rodent impaction graft

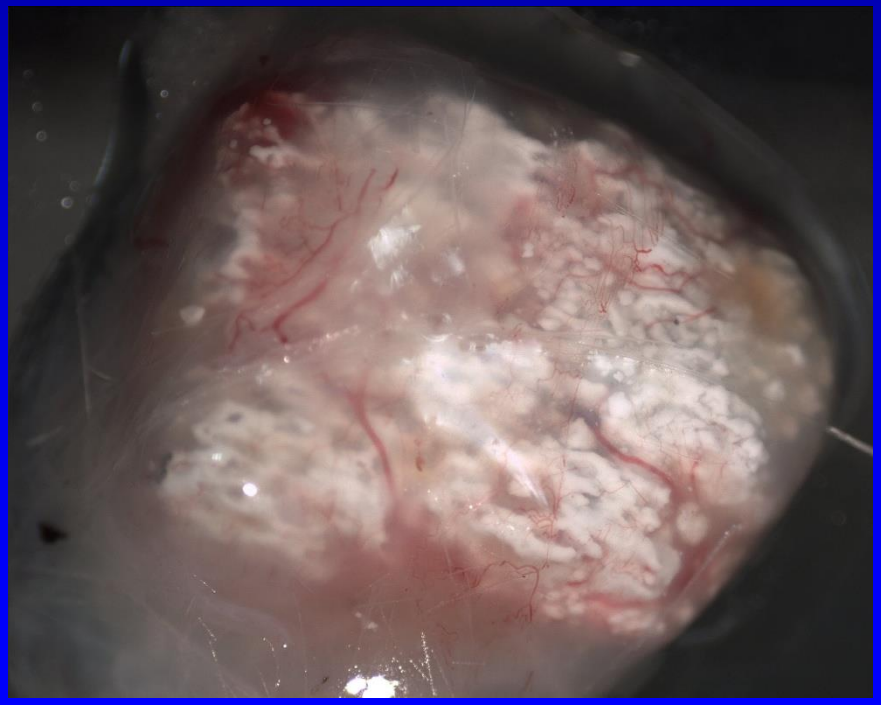
small animals for genomics

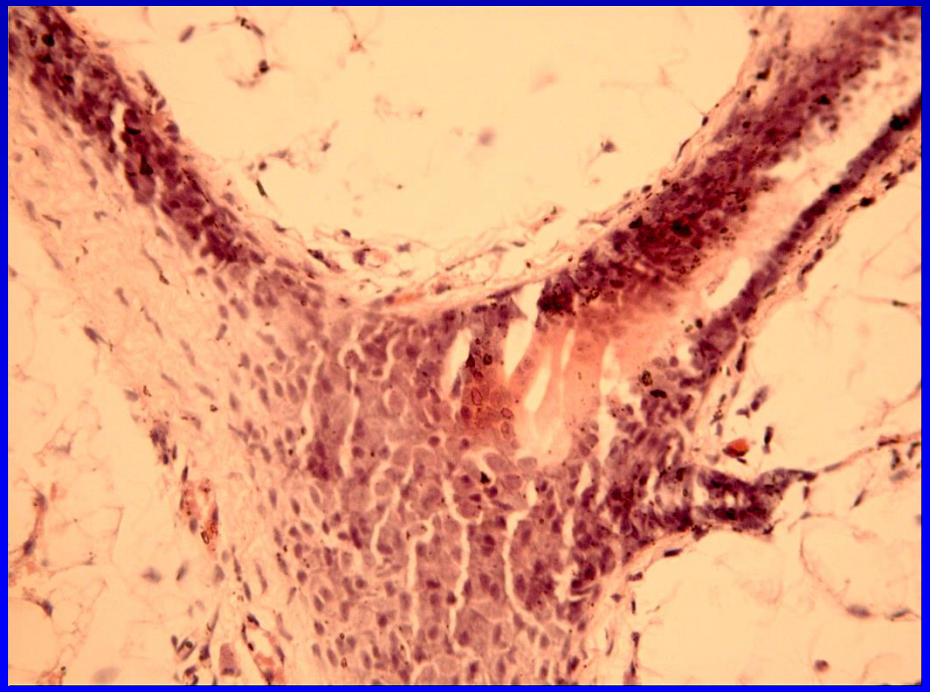
large animals for mechanics

The Mouse Fracture Model

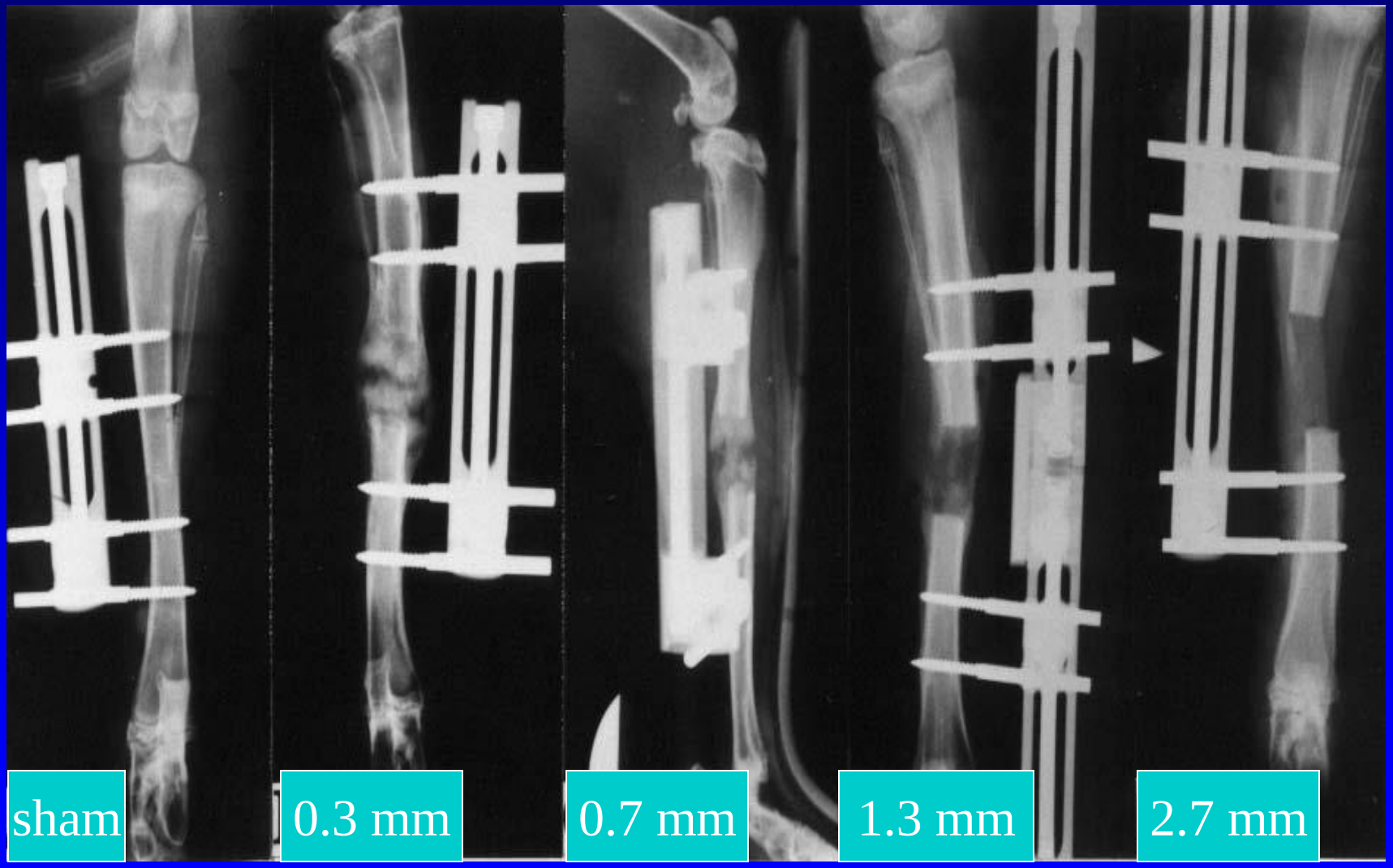
(Li, CONNOLLY, et al, JBMR, No 5, 2002)







Radiography

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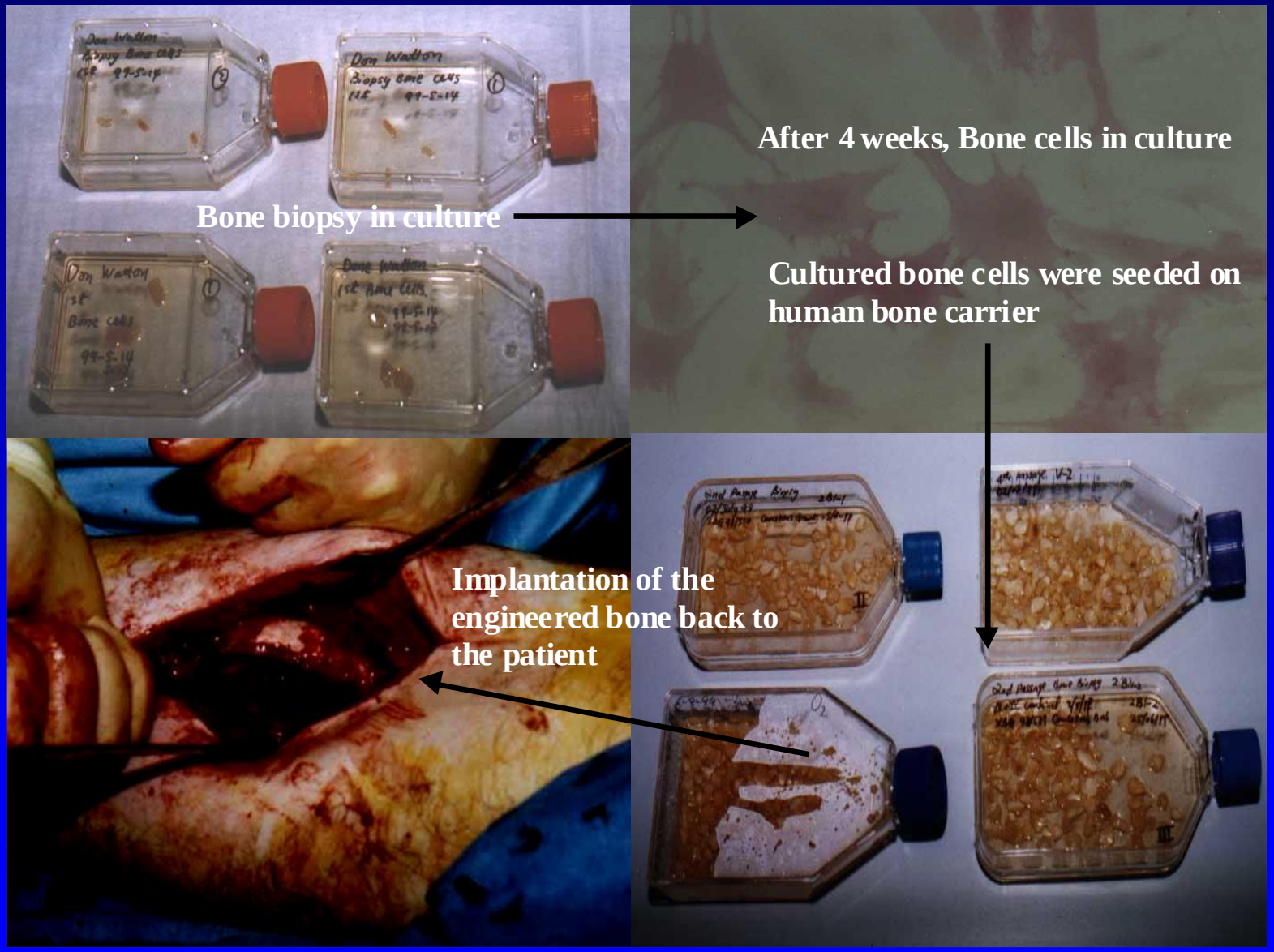
Human models

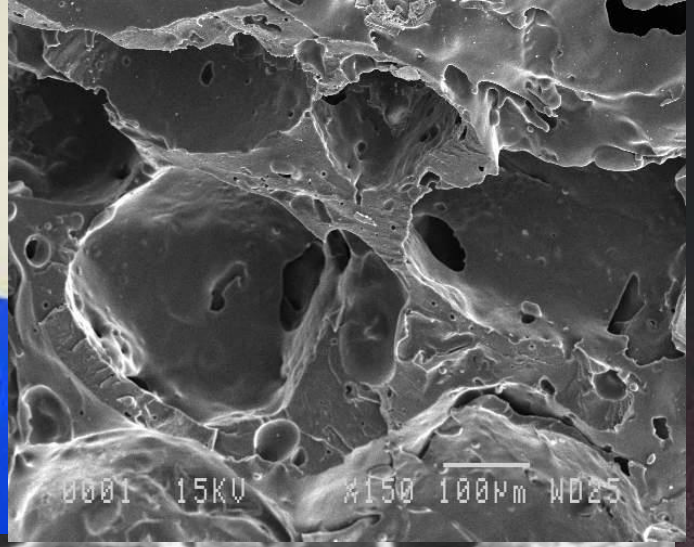
- high energy tibial shaft fracture
- tibial distraction osteogenesis
- impaction graft in revision hip
- post-osteomyelitic cavity

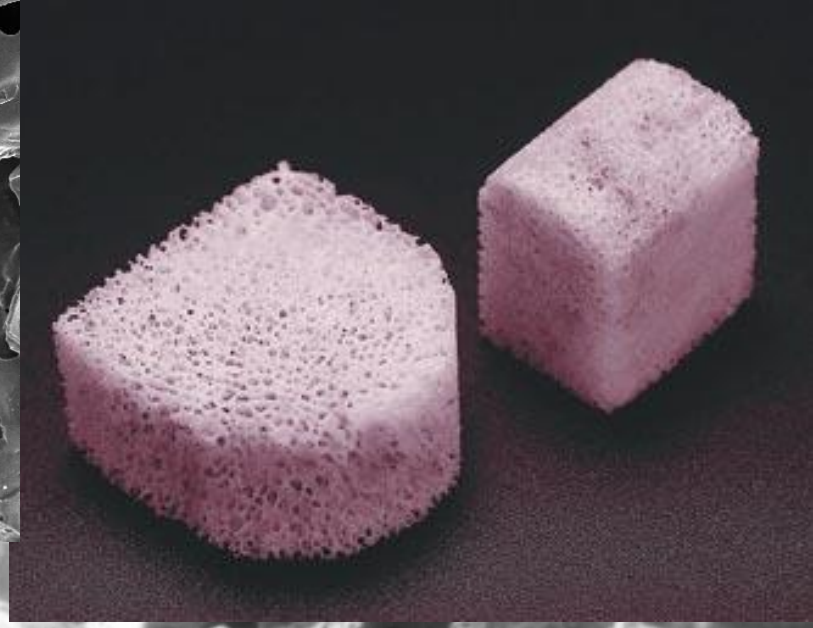
Enhancement of in-vitro human osteoblast growth by culture with endothelial cells - towards a living autologous bone graft

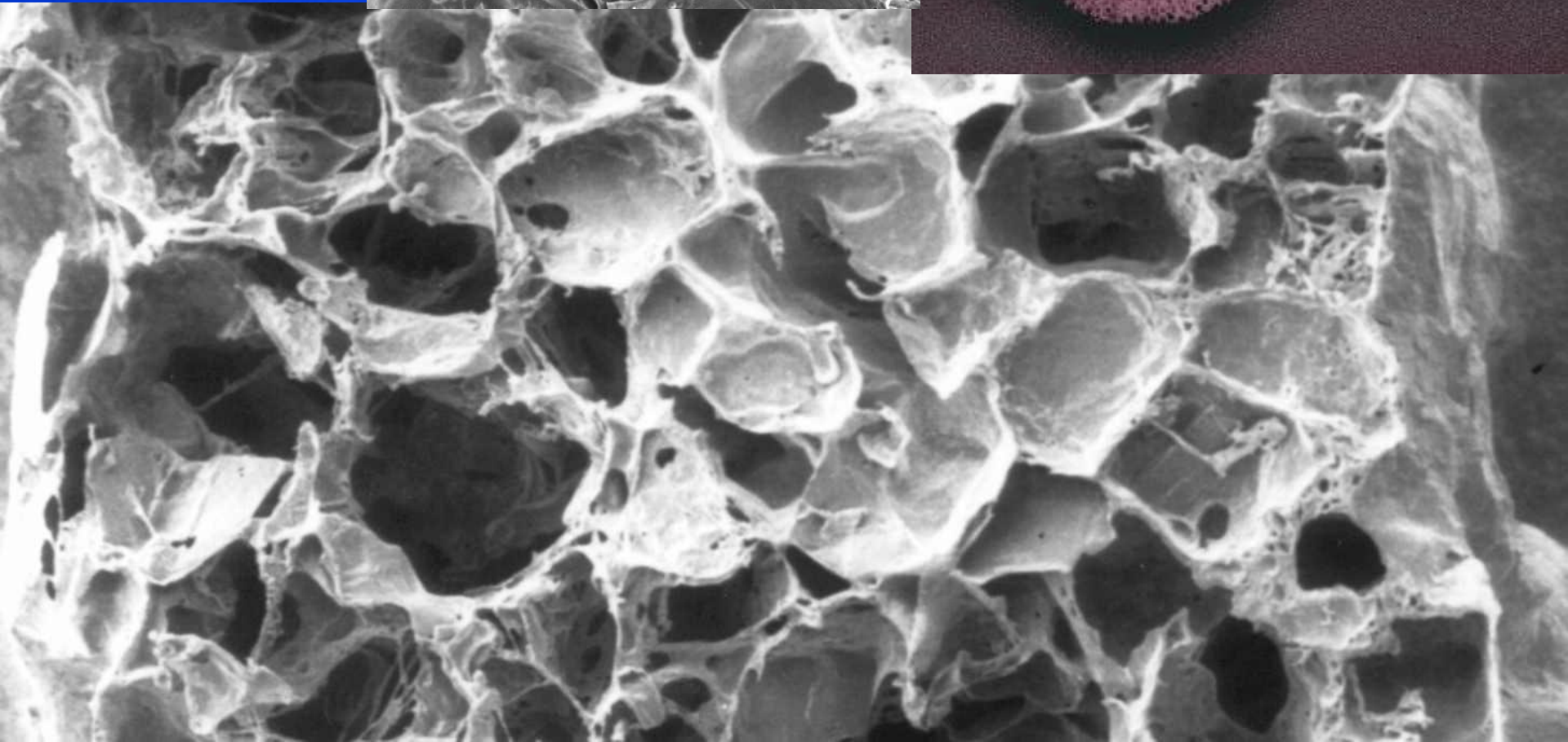


Matrix/bio-materials design

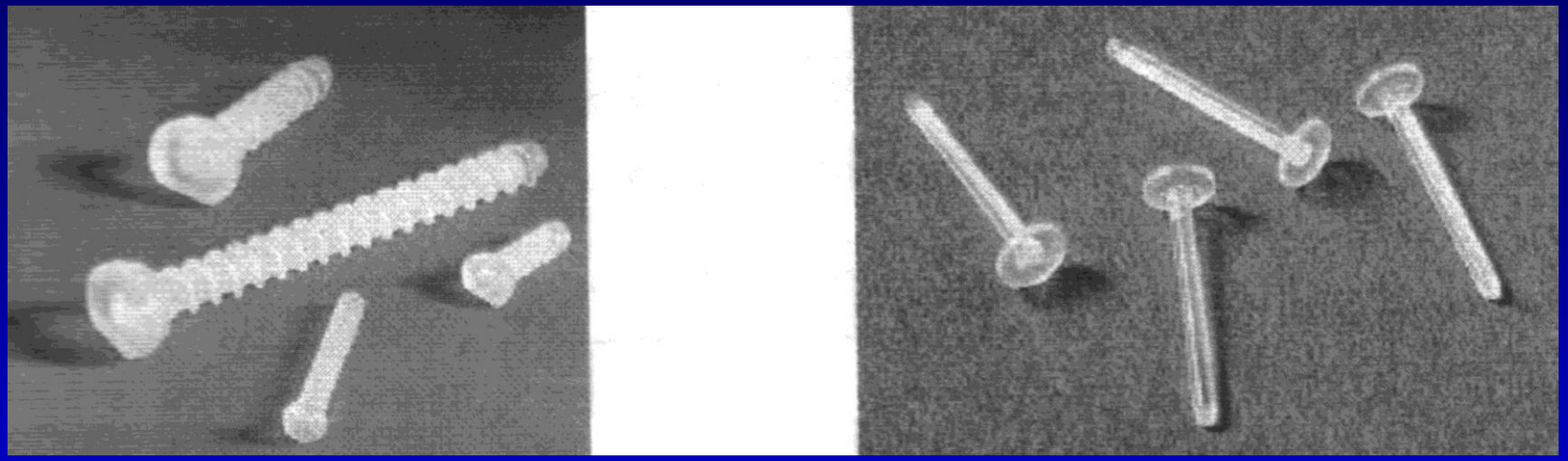
- material
  - ↗resorbable polymers
  - ↗plastics
  - ↗Ca ceramics
- geometry, porosity
  - strength vs vascularisation
  - ↗lost wax
  - ↗rapid prototyping


0001 15KV X150 100µm WD25





Bioabsorbable Polymers



Smart screw
Bionx implants
PLLA
Poly-L-Lactide

Smart Tack
Bionix implants
PGA
Polyglycolide

Vascularisation is estimated to average between 0.09-0.25 mm /day (Zawicki et al, 1981)

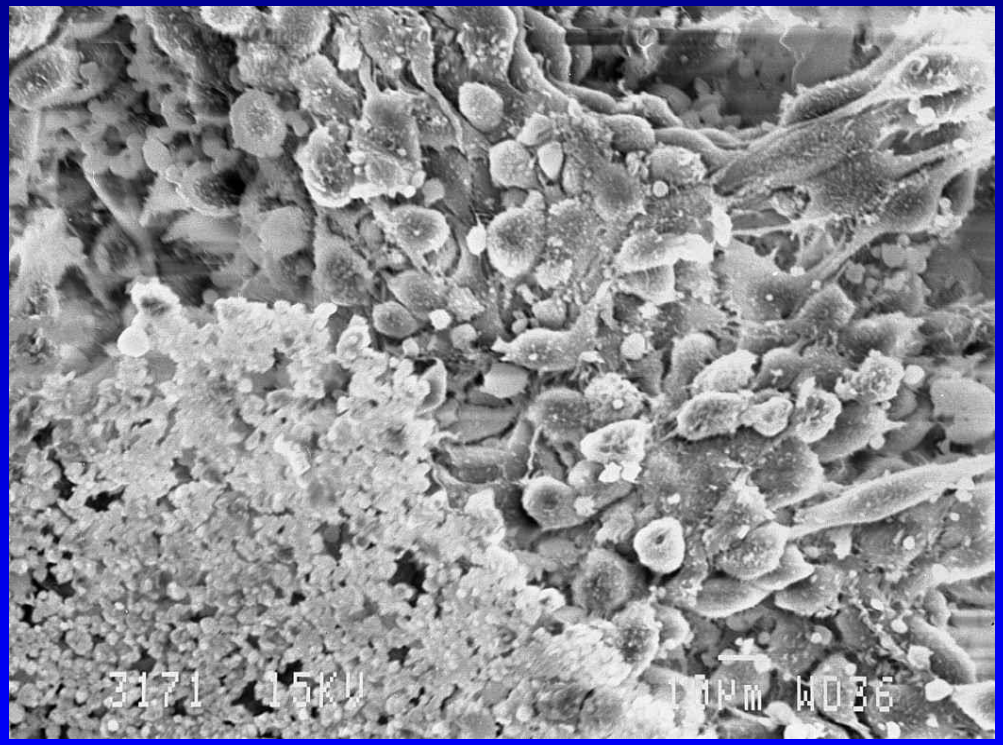
Pore sizes

fibrovascular ingrowth: < 15-50 μm

osteoid formation: 50-150 μm

encourage ingrowth of
of mineralised bone: > 150 μm

A Challenge for Tissue Engineering



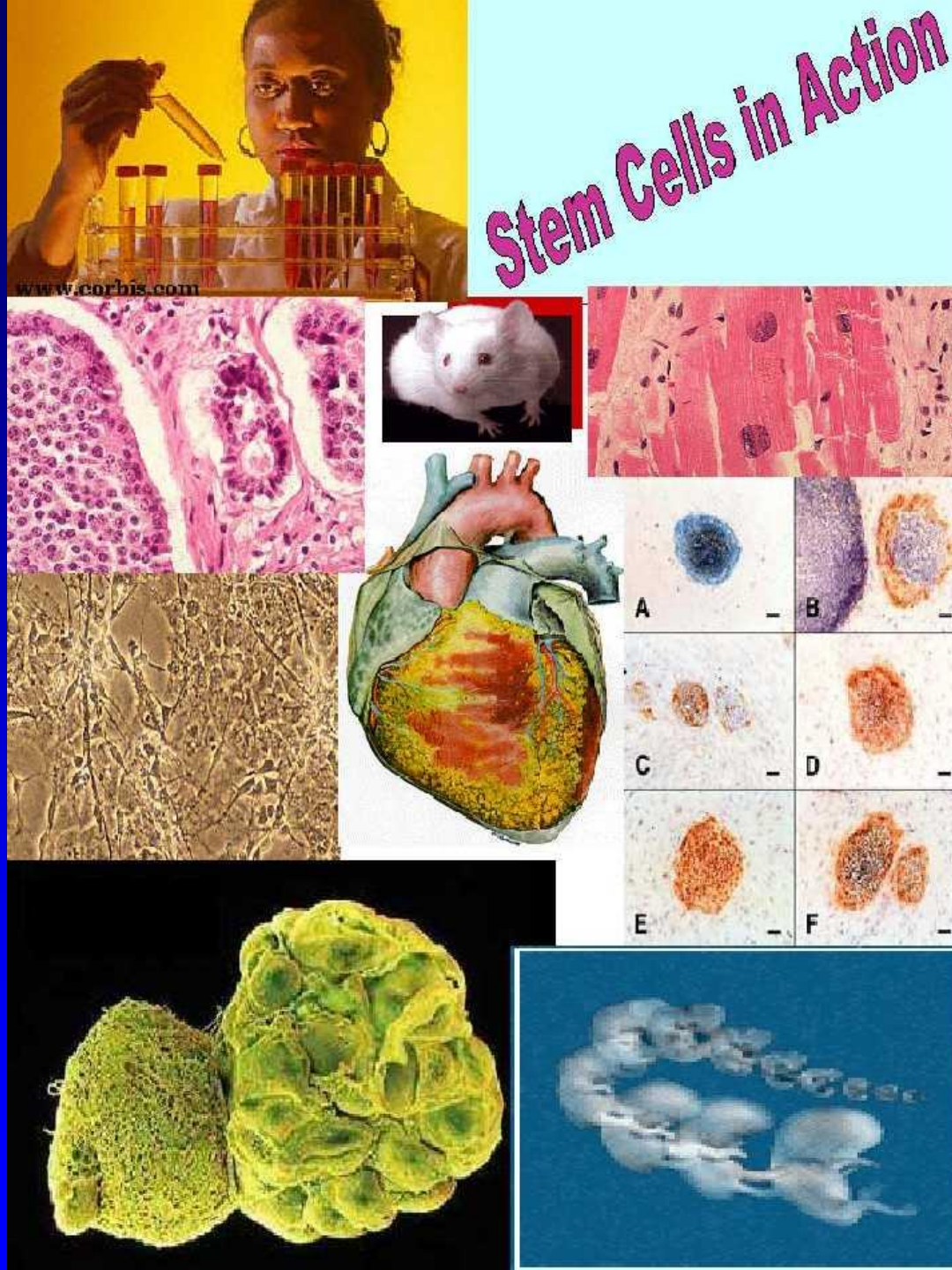
Interactions between biomaterials and cells need to maintain cell adhesion and **phenotype** within the engineered tissue.

Future of Tissue Engineering

1. Stem cell application
2. Intelligent biomaterials
3. Gene therapy
4. Functional tissues

Future of Tissue Engineering

1. Stem cell application

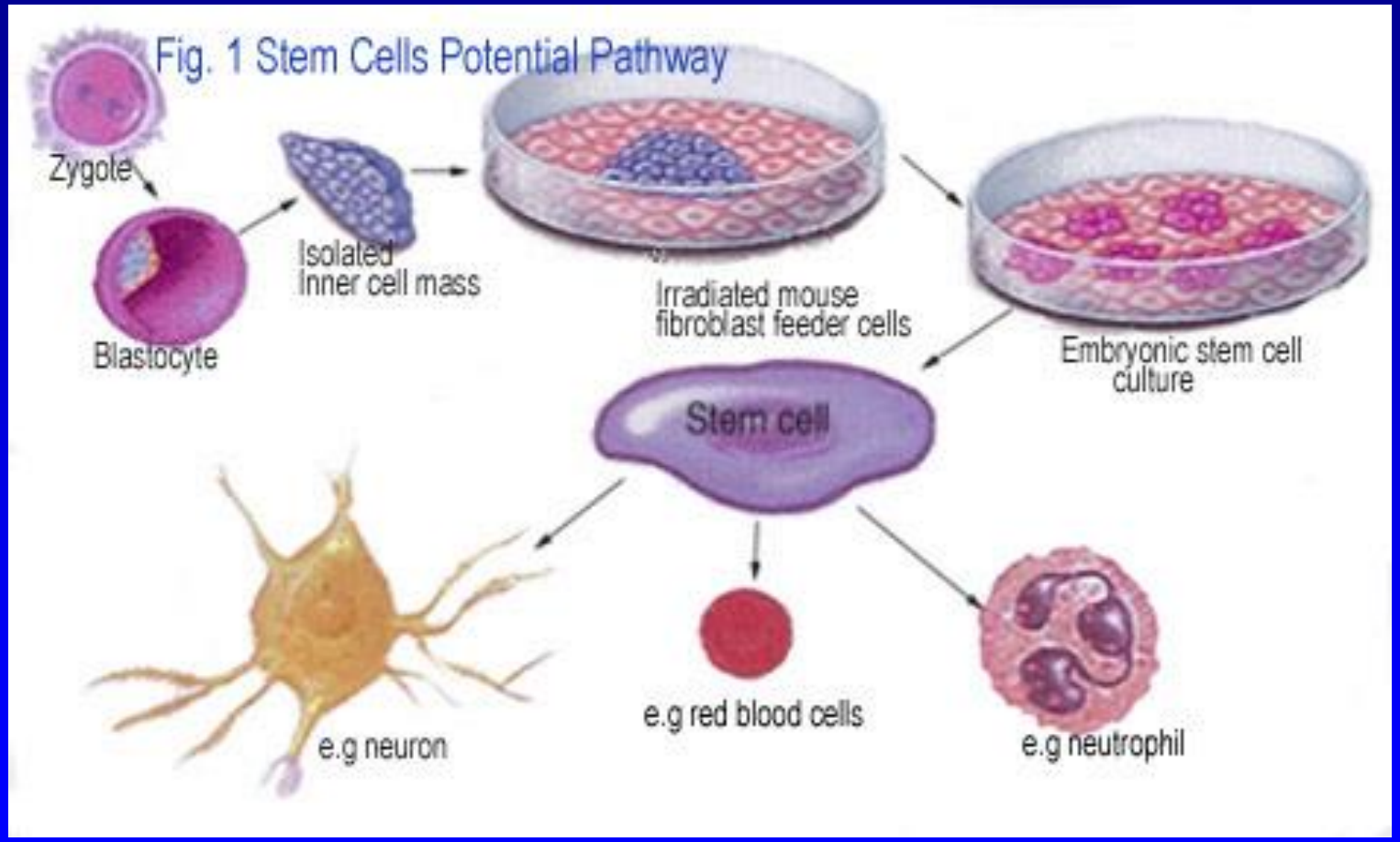


Future of Tissue Engineering

2. Intelligent biomaterials

Future of Tissue Engineering

Gene therapy and Functional tissues



Summary

- Cell, materials and their combinations
- Medical/biology/engineering team
- Learn and use natural routes
- Devise protocols and test by RCT
- Stem cells and vascularisation the key


Thank You !!