Stem Cells - I

Adult vs Foetal Stem Cells

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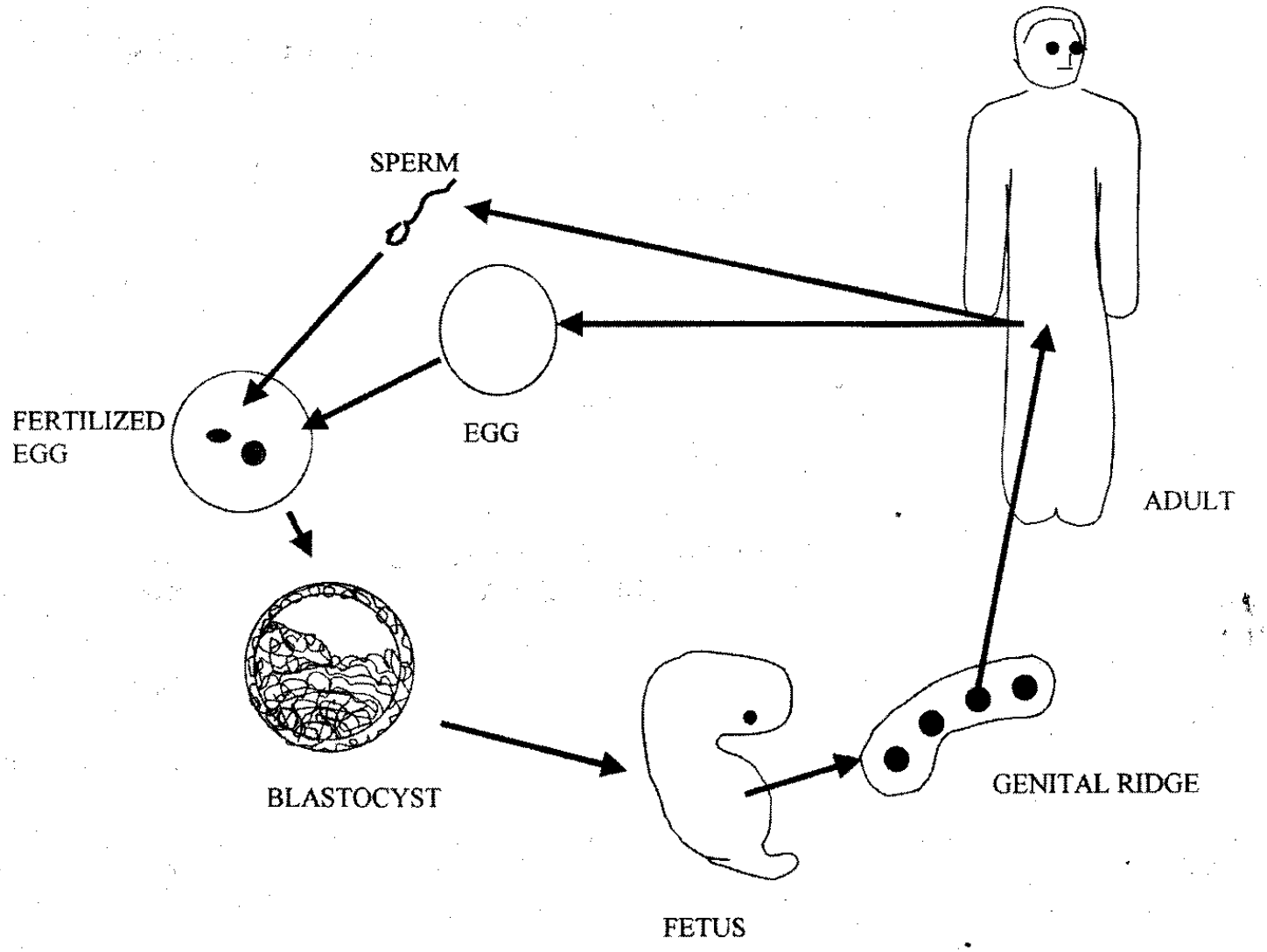
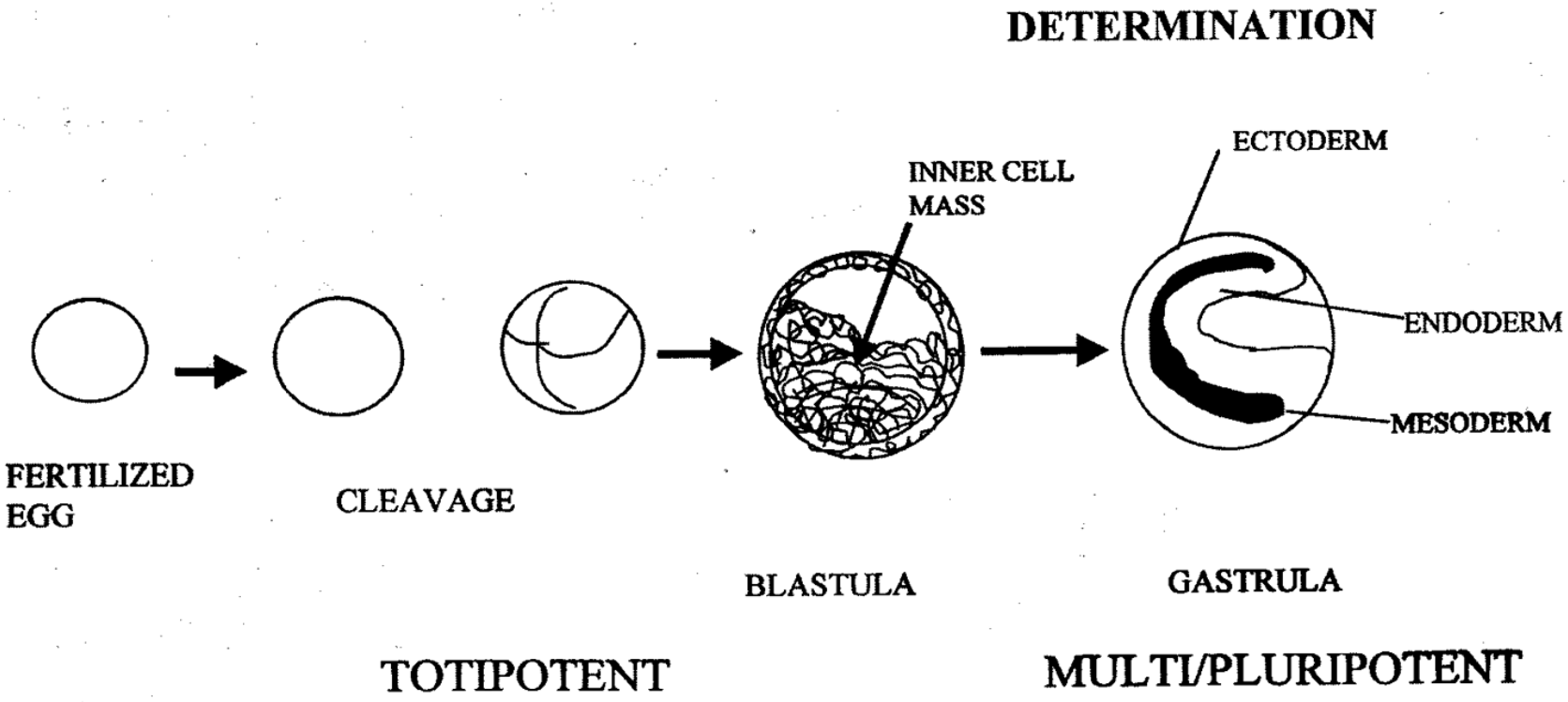
Life Cycle

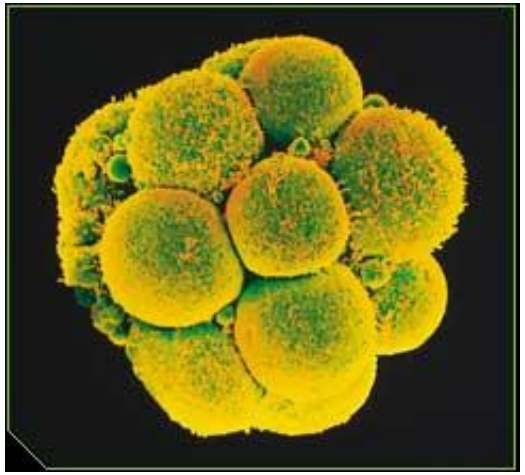

SPERM
FERTILIZED EGG
EGG
ADULT
BLASTOCYST
GENITAL RIDGE
FETUS

Stem Cell Life Cycle

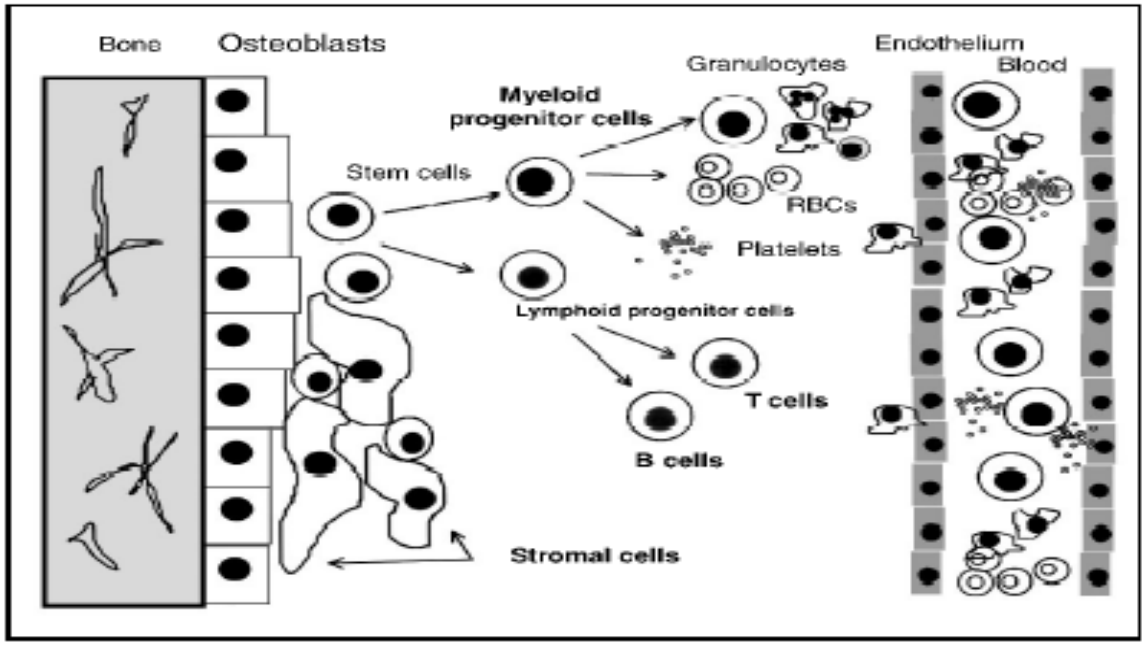


What is stem cell ?

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



- **Embryonic stem cells**
- **Adult stem cells**



Two distinct systems ?:

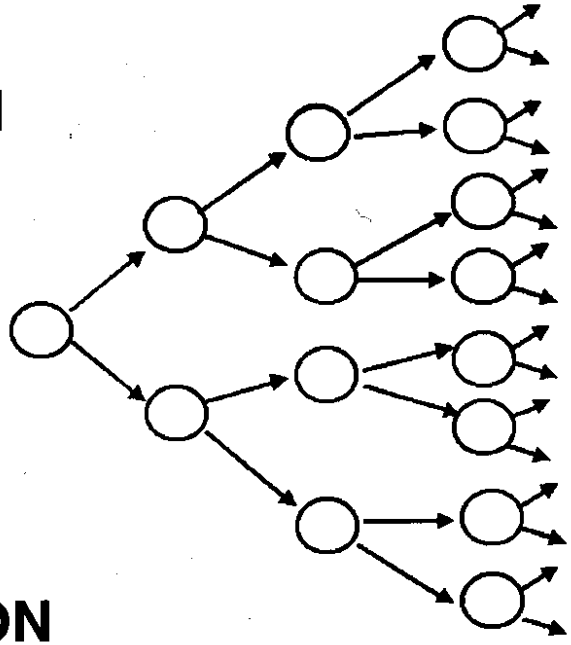
1.Hematopoietic stem cells (HSCs)

2.Mesenchymal stromal cells (MSCs)

Stem Cell Division

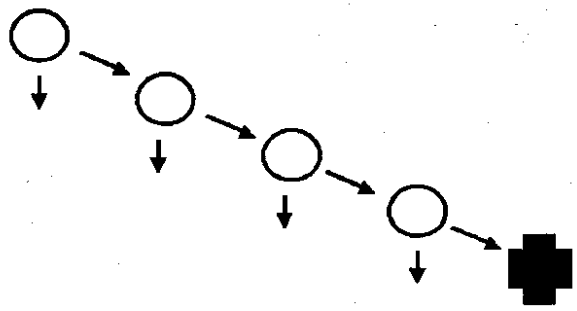
SYMMETRIC DIVISION

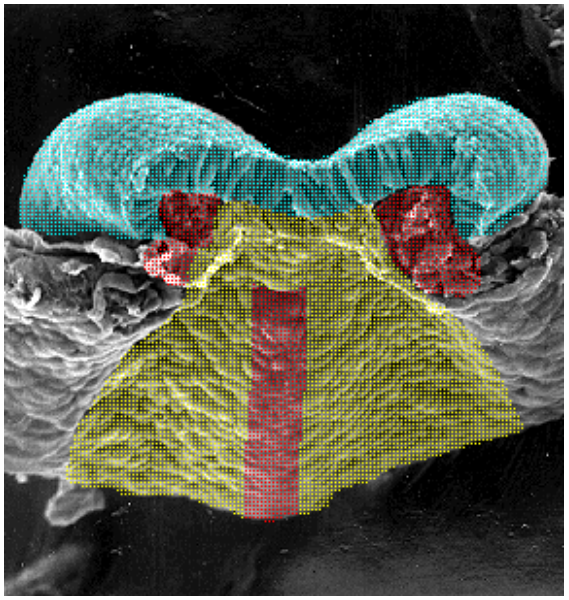
THE EMBRYO STEM CELL DIVIDES TO YIELD TWO IDENTICAL TOTIPOTENT DAUGHTER CELLS



ASYMMETRIC DIVISION

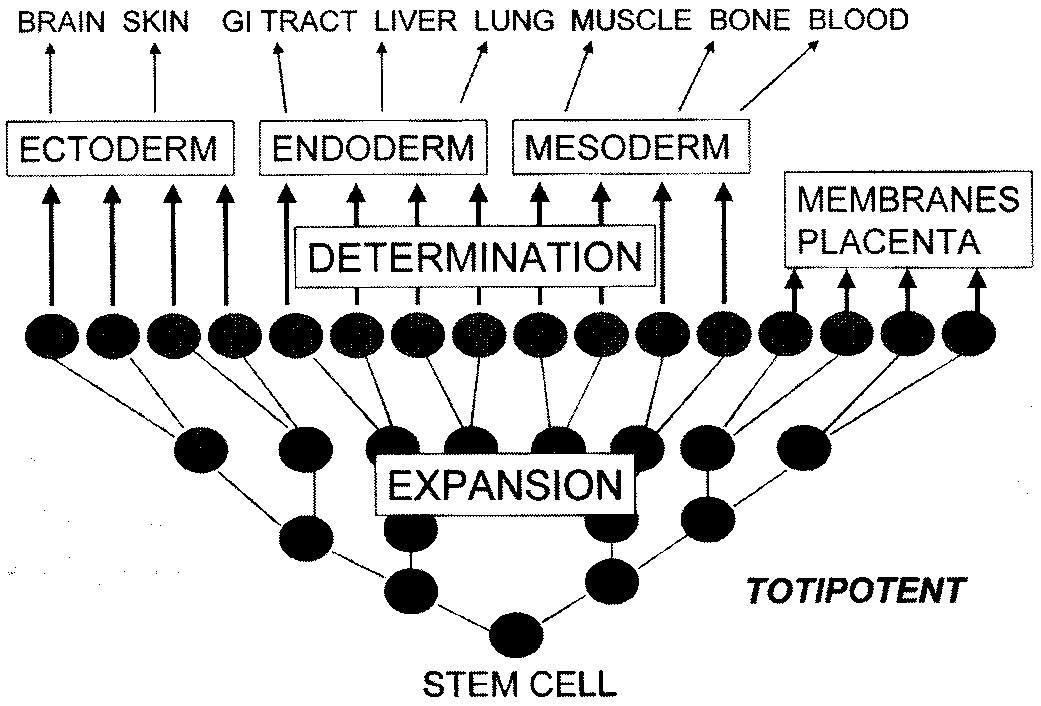
THE TISSUE PROGENITOR CELL GIVES RISE TO ONE DAUGHTER CELL WHICH REMAINS A PROGENITOR CELL AND ONE DAUGHTER CELL WHICH BEGINS THE PROCESS OF DIFFERENTIATION LEADING TO TERMINATION



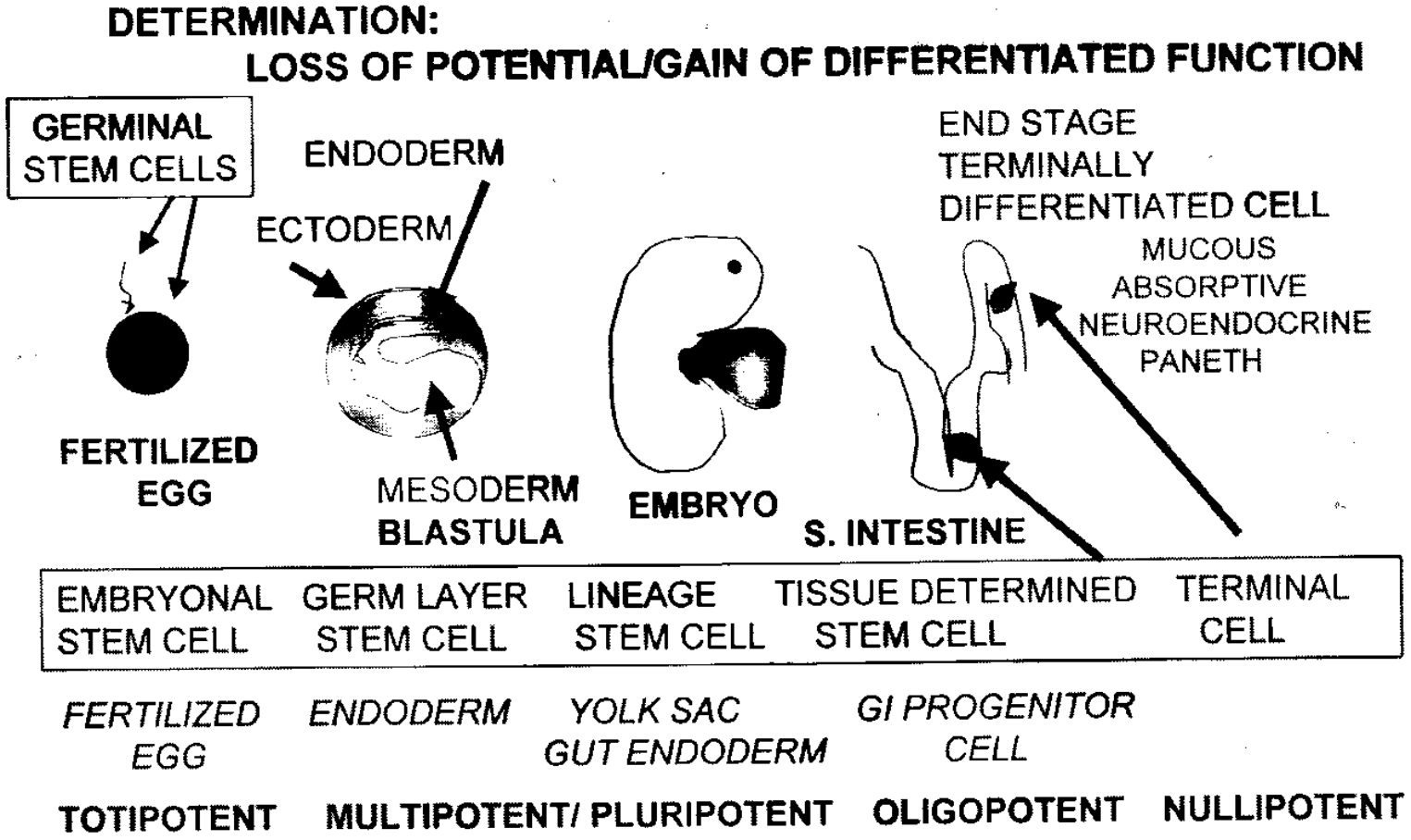


- **Ectoderm**: CNS and Brain
- **Mesoderm**: Skeletal Systems
- **Endoderm**: Blood system and other internal organs

Embryonic Stem Cell Differentiation



Stem Cell Determination



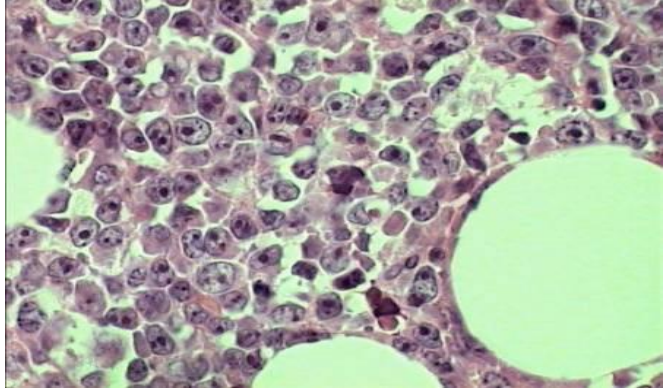
Postulated Turnover Rates and Potentials of Progenitor Cells in Some Adult Organs

Organ	*Replacement time*	*Potential*
Bone marrow (polys)	1–2 days	Multi/pluripotential (?toti)
GI lining	2–4 d	Quadripotential (four types)
Skin	2 wk	Bipotential
Liver	1–2 yr	Bipotential
Brain (neurons)	Lifetime	Unipotential[a]

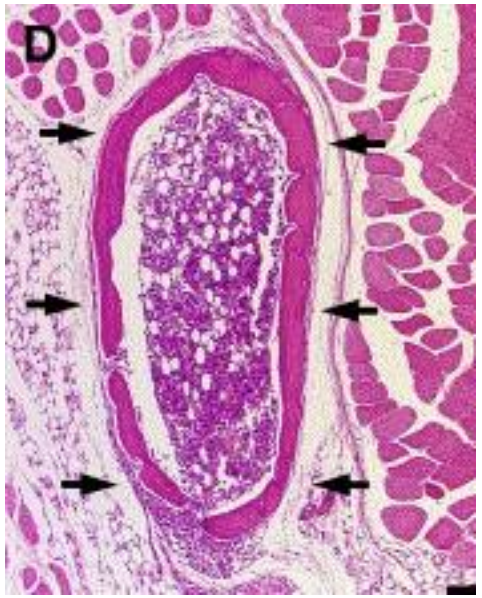
Sources of MSCs

- Bone marrow
- Adipose tissue
- Periosteum
- Skeletal muscle
- Adult peripheral blood
- Umbilical cord blood
- Bone tissue
- Amniotic fluid
- Spleen
- Dermis
- Vascular pericytes

Bone marrow



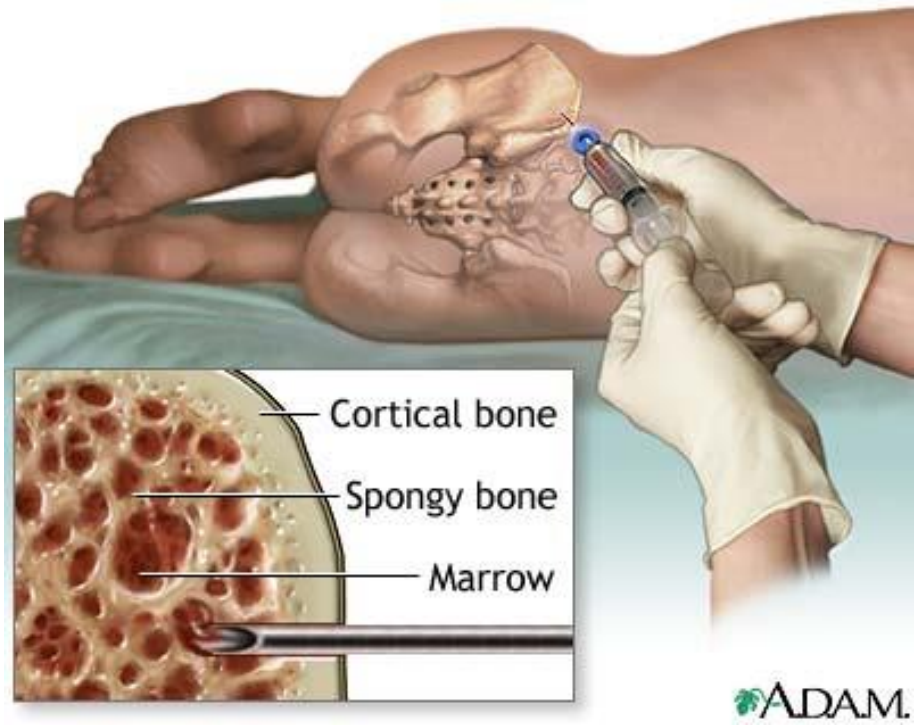
- First source identified
- Relatively high numbers present
  - (one in 3400)
- Differentiated osteoprogenitors present also
- BM-MSCs generate bone *in vivo*
- Most researched and established
- More understood
- Human clinical trials



Bone formation *in vivo* using BM-MSCs

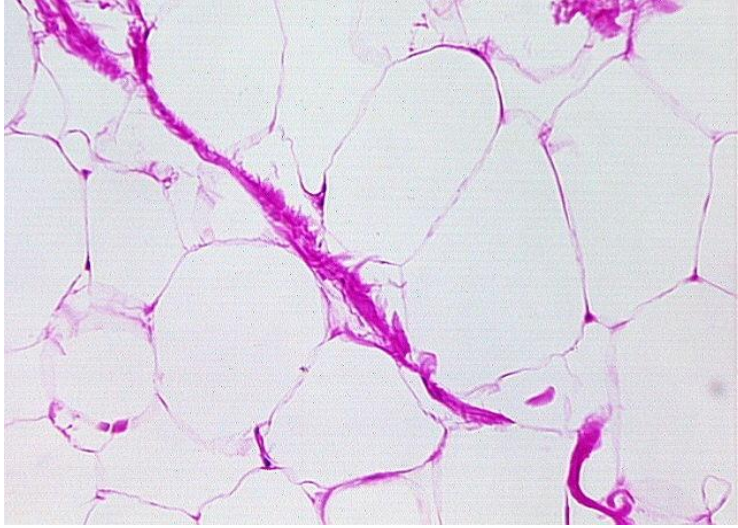
BM aspiration – a disadvantage

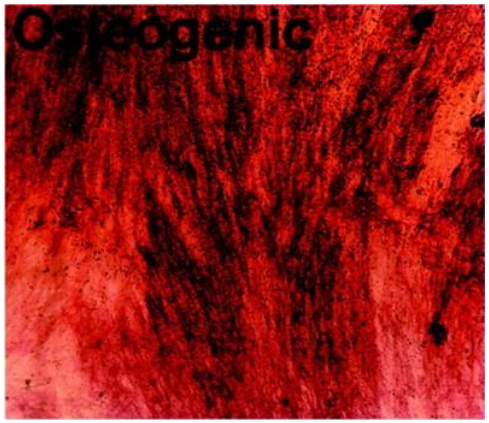
- 20-30 min
- 1 hour supine afterwards
- Local anaesthetic
- Pain



Adipose tissue

- Infrapatellar fat pad & lipoaspirate
- Lower osteogenic potential than BM
  - 75% OB differentiation possible
- Bone formation in rats
- Slightly properties different to BM-MSCs

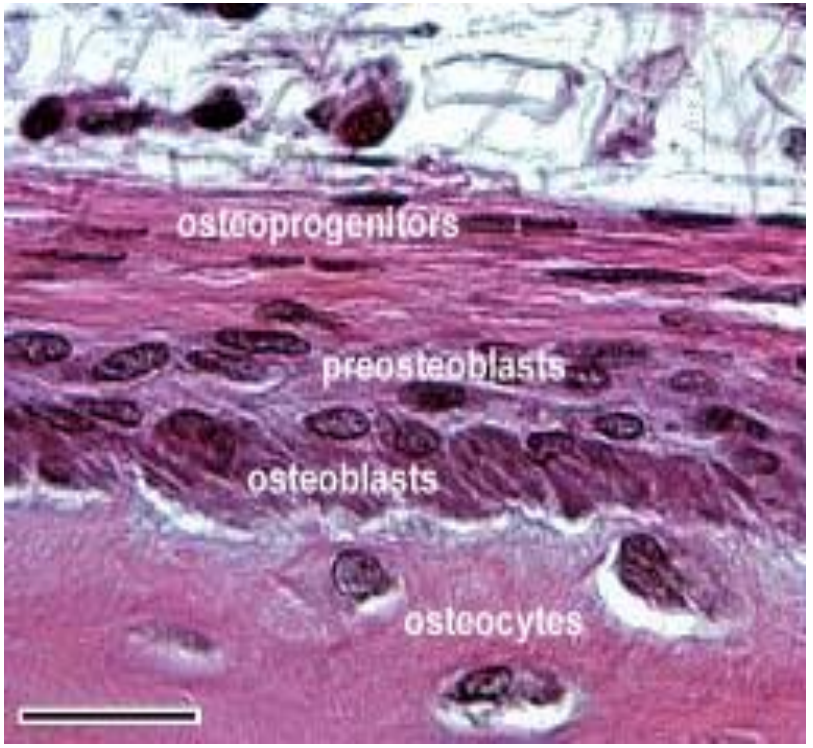




Cells expressing calcium phosphate differentiated from adipose derived MSCs

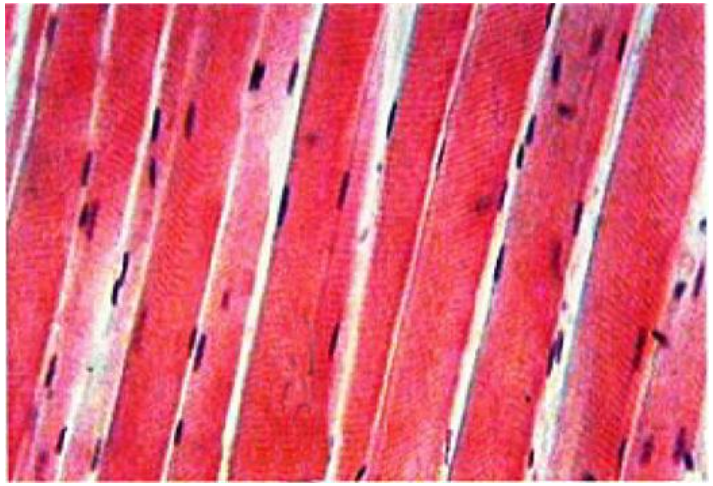
Periosteum

- Outer layer differentiate into OBs
- Grafting
- 100% OB potential
- *In vivo* bone formation



Skeletal Muscle

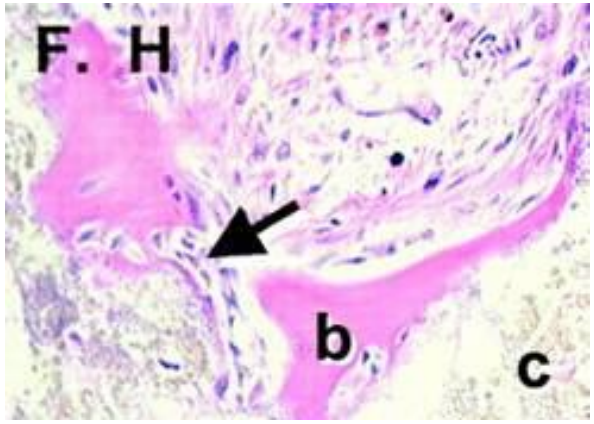
- Ectopic bone formation
- Satellite cells
- Gene therapy
- Levey *et al* :
  - 70% expressed ALP
  - Osteocalcin present



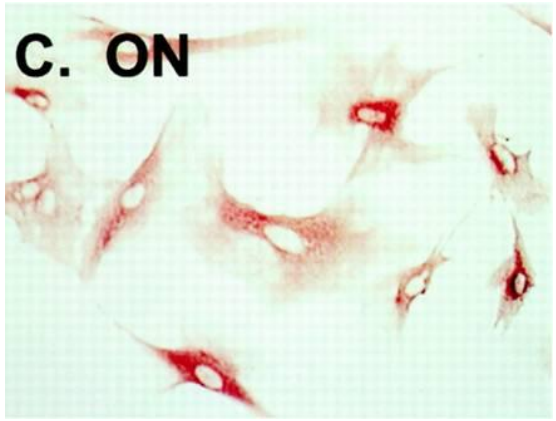
Muscle satellite cells

Adult Peripheral Blood

- One MSC for every $2x10^9$
- Bone formation observed when osteogenic cells transplanted into mice

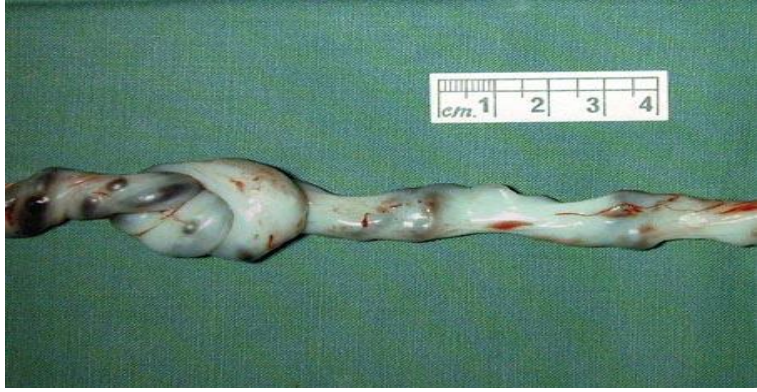


Human bone formation in guinea pig from blood MSCs

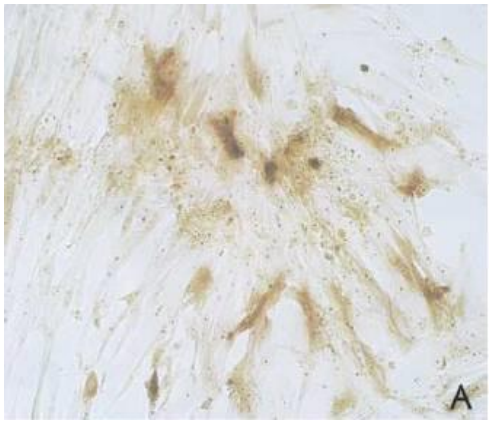


Differentiated human blood MSCs expressing osteonectin

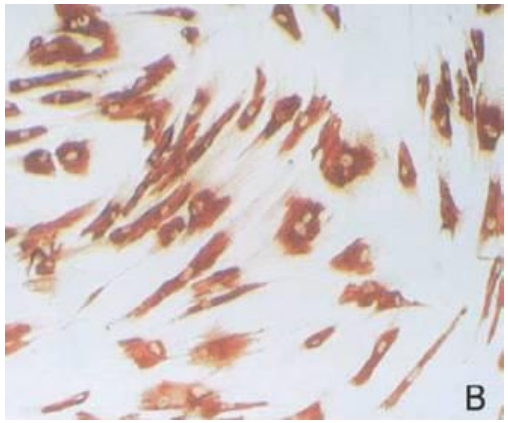
Umbilical Cord Blood (UCB)



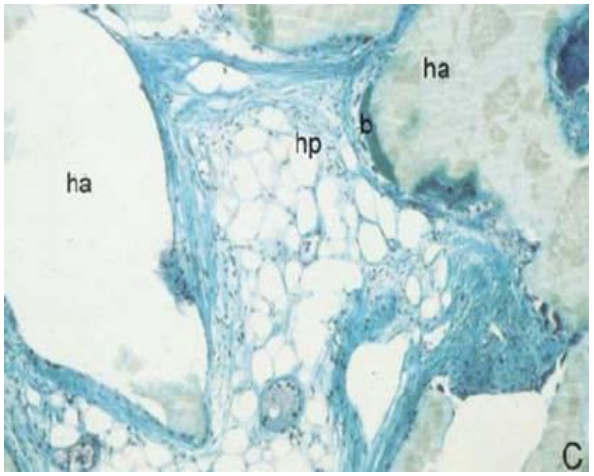
- MSCs have been cultured from full term UCB
- More stroma tissue than bone when transplanted



Differentiated UCB MSCs expressing ALP *in vitro*



Differentiated UCB MSCs expressing osteocalcin *in vitro*



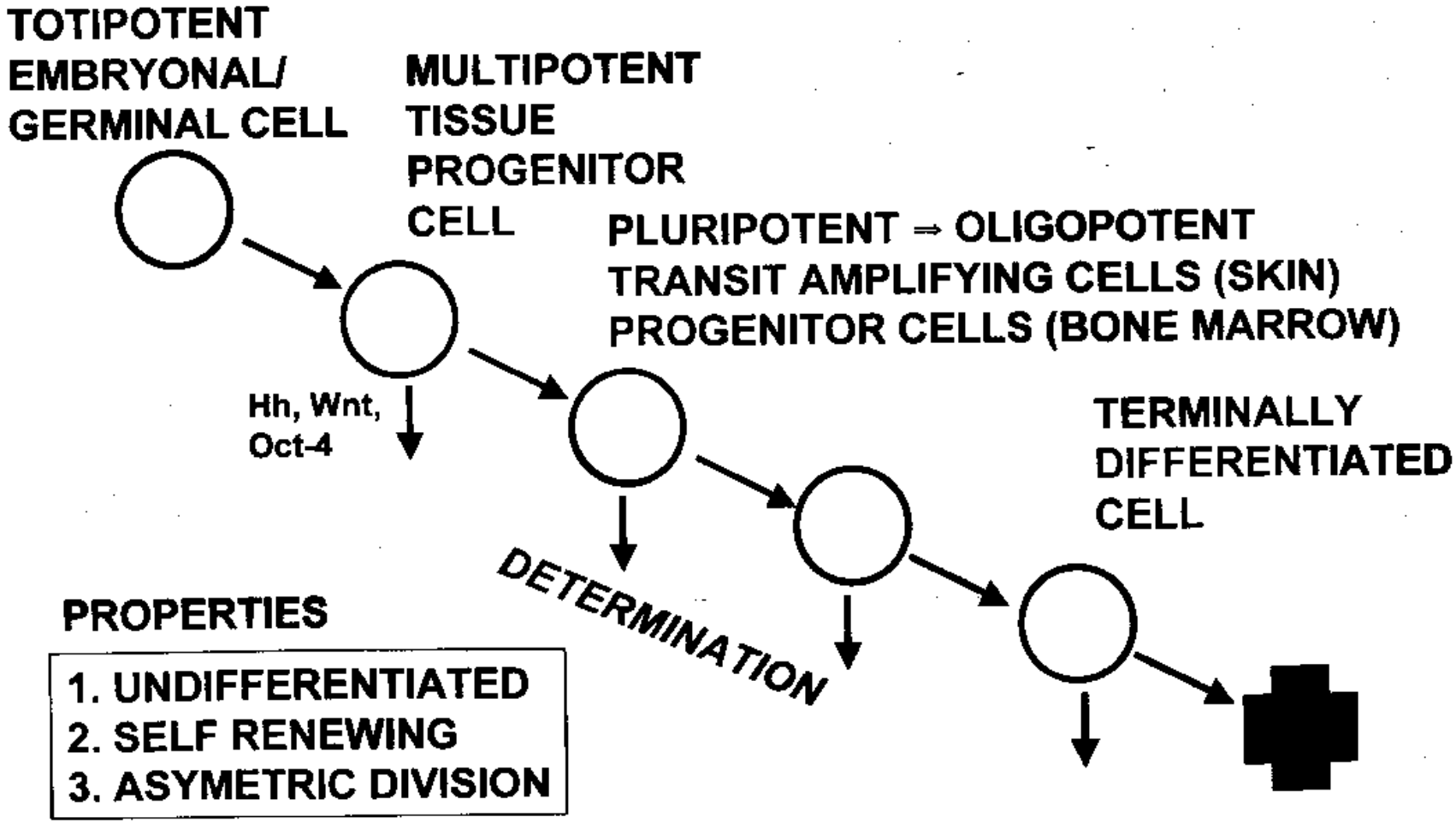
Bone production by UCB MSCs *in vivo*

Other sources

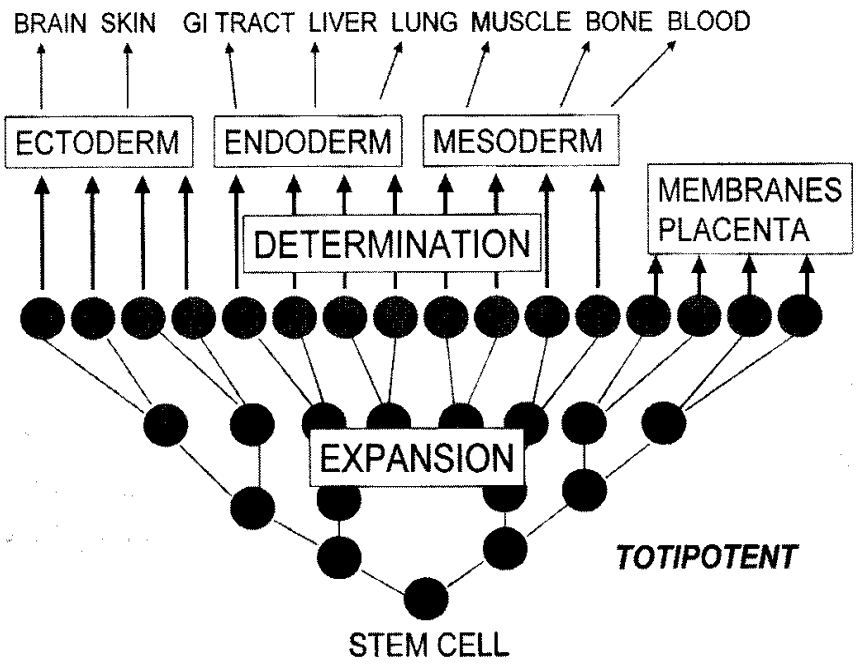
- Amniotic fluid
  - MSCs greater expansion potential than BM-MSCs
  - Allogeneic transplant, limited supply
- Spleen
  - Observed in rats (one study)
  - Difficult removal
- Dermis
  - Two studies one successful, one not
  - Limited research

- All sources classed as “other” have a limited therapeutic potential

Summary: Stem Cell Life



Embryonic Stem Cell Vs. Adult Stem Cell



- **Ethical issues of the use of ESC.**
- **Not clear if ESC will respond to signals derived from the microenvironment of adult organ/tissue.**
- **Potential risks of ESC.**

- **Increasing evidence show that adult stem cells may be just as good, or even better than ESC.**
- **"Seed and Soil" hypothesis; -- cell potential, environment, cell-matrix, cell-cell, growth factors**

Summary

- There are a wide variety of stem cells sources.
- Adult stem cells are safe and easy to collect.
- Bone marrow is the most established, best understood, and most reliable source.
- Adipose tissues and cord blood derived MSCs also have therapeutic potentials.
- Adult peripheral blood is most promising source.

Stem Cells - II

Differentiation Potentials of Mesenchymal stem cells

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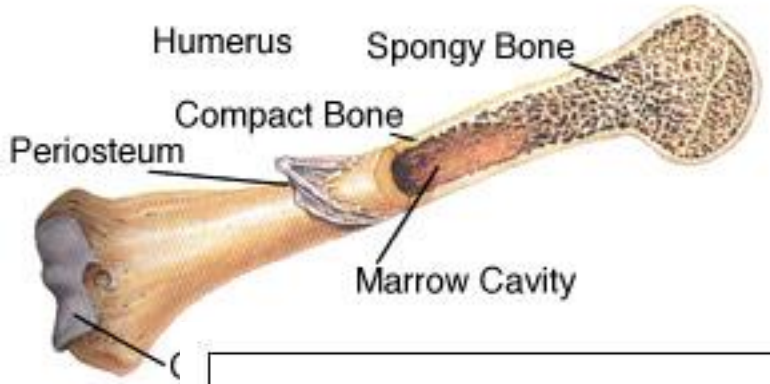
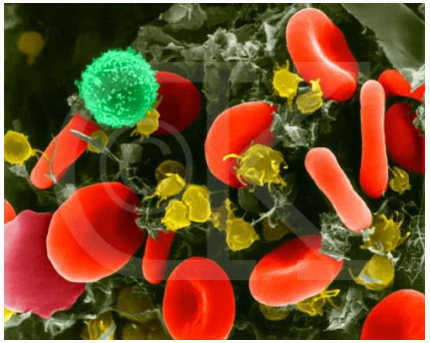
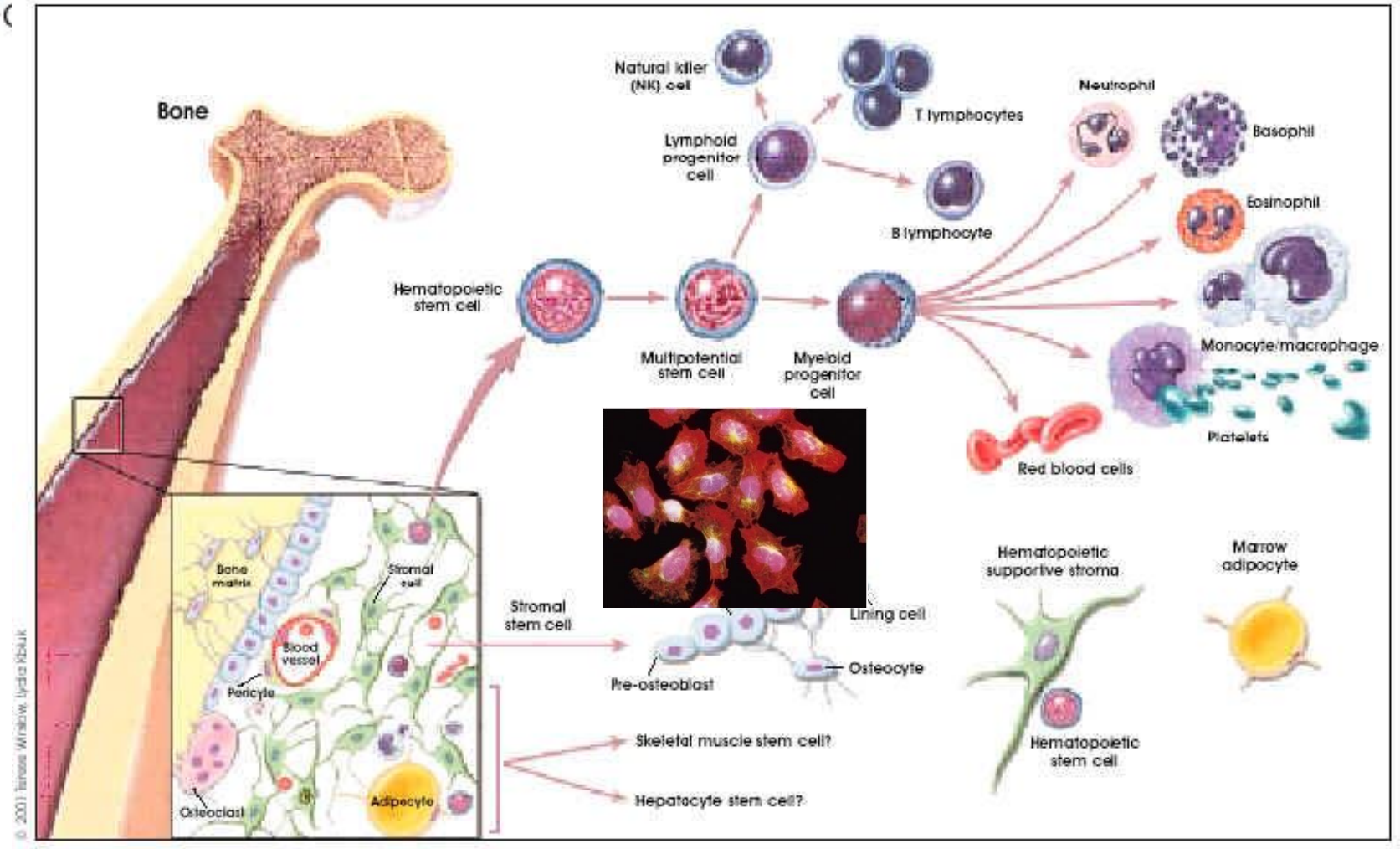




Multiple potentials of bone marrow MSCs

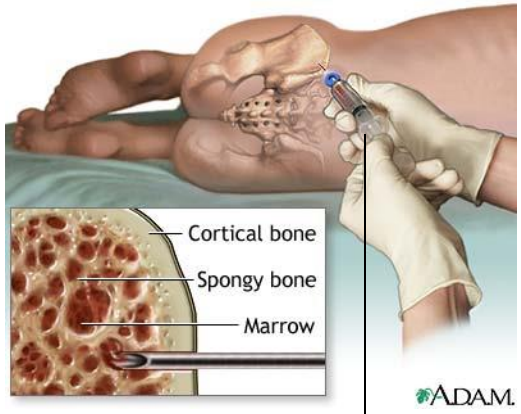


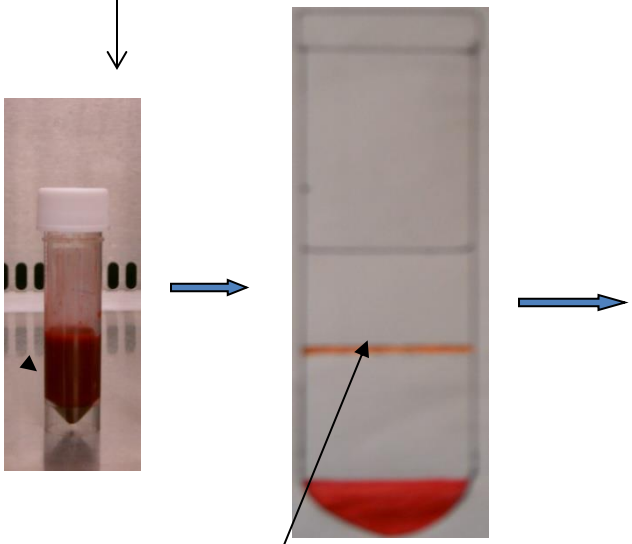
BM-derived CFU-Fs

CFU-F colony

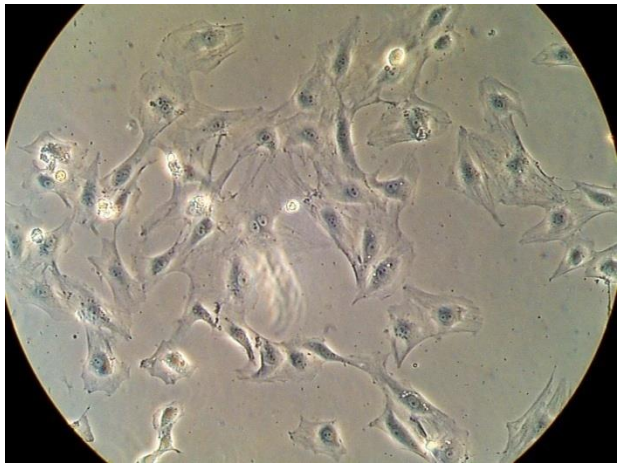
- Bone
- Cartilage
- Adipose tissue
- Fibrous
- Myelosuppotive stroma

BM-derived CFU-F



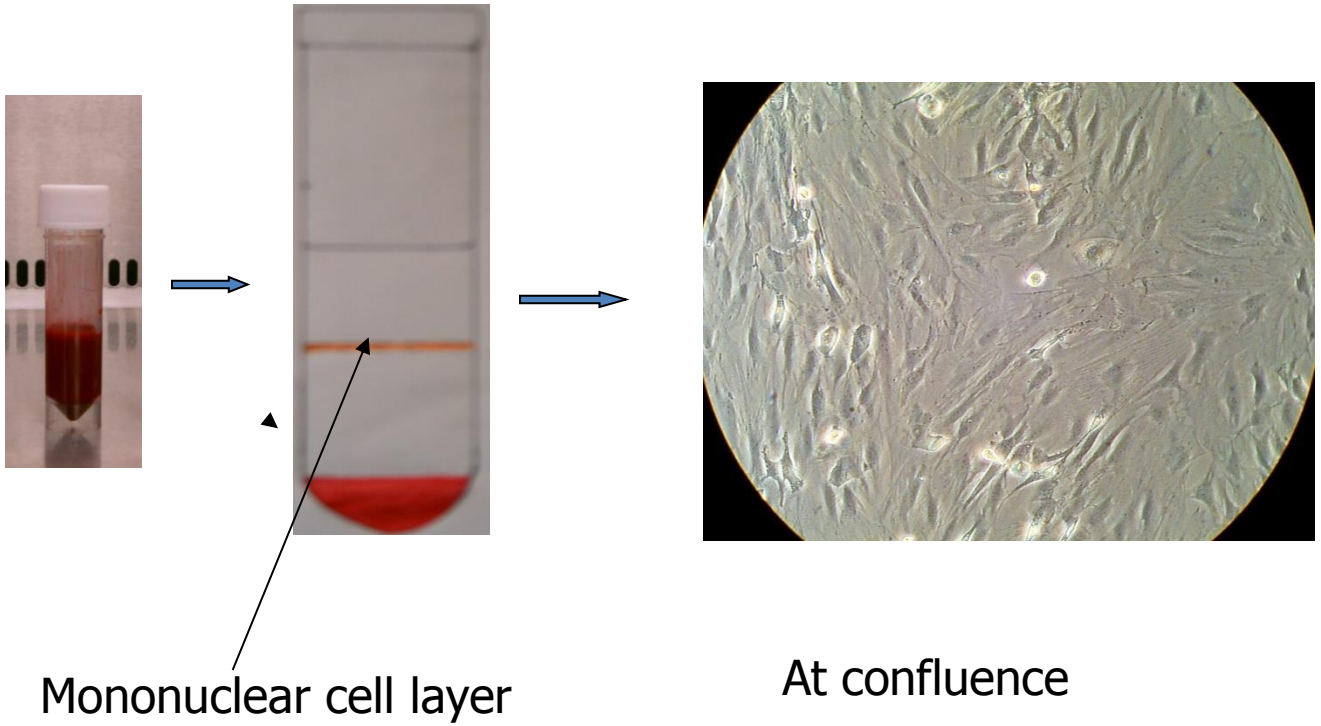


Mononuclear cell layer

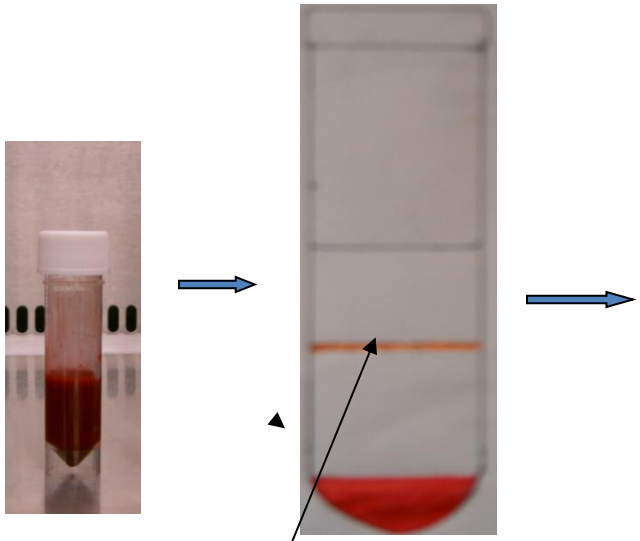


12-14 days

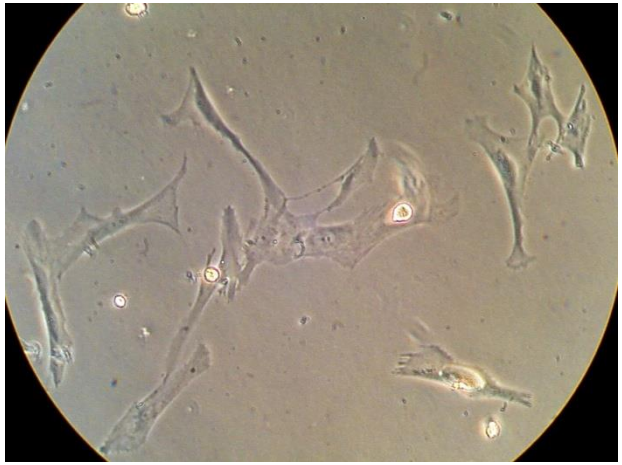
BM-derived CFU-F



BM-derived CFU-F

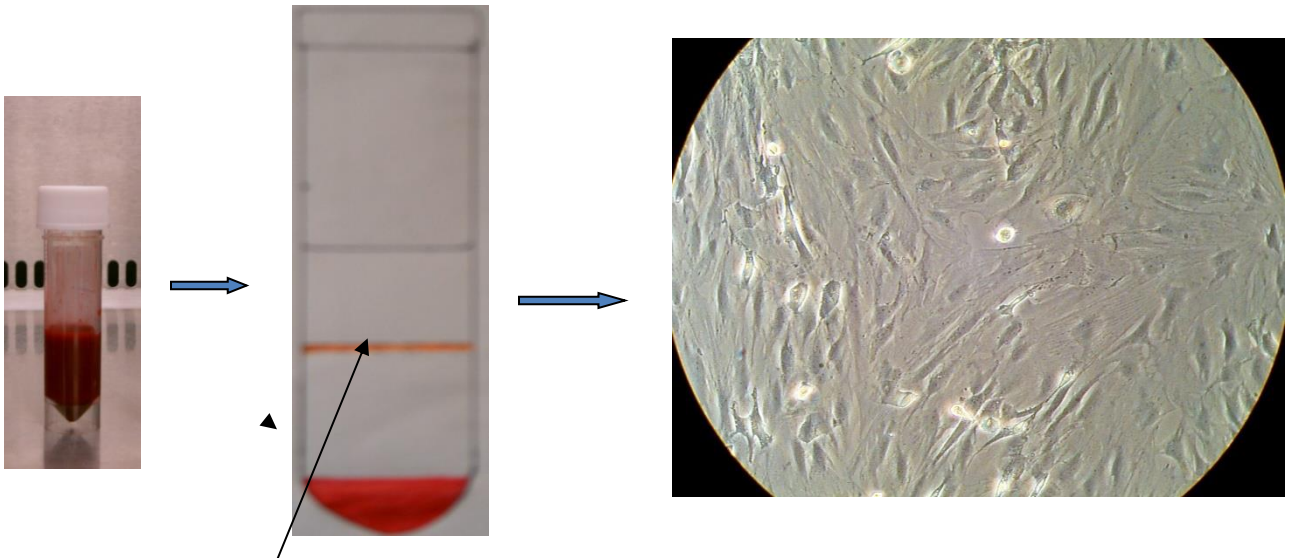


Mononuclear cell layer



Passage 1

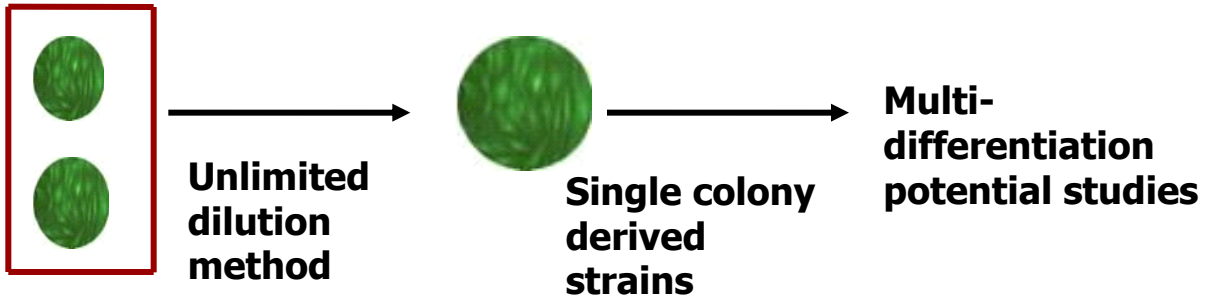
BM-derived CFU-F



Mononuclear cell layer

At confluence

Studies of multi-differentiation potentials of MSCs



Osteogenic inductive condition

- DMEM (low glucose)
- 10% FBS
- 100IU/ml penicillin
- 100μg/ml streptomycin
- 2.5μg/ml fungizone
- 10^{-7}M dexamethasone
- 0.2mM ascorbic acid-2-phosphate
- 10mM β-Glycerophosphate

Adipogenic inductive condition

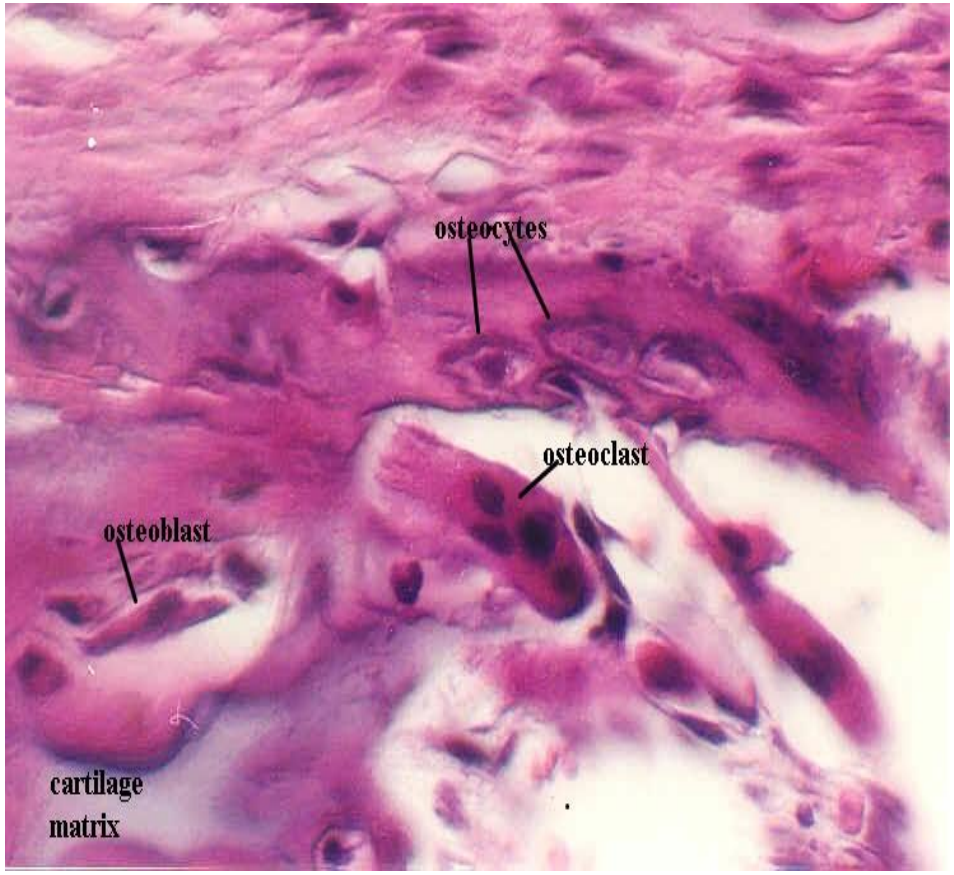
- DMEM (high glucose)
- 10% FBS
- 100IU/ml penicillin
- 100μg/ml streptomycin
- 2.5μg/ml fungizone
- 10^{-6}M dexamethasone
- 0.50mM IBMX
- 50μM indomethacin

Chondrogenic inductive condition

- DMEM (high glucose)
- 100IU/ml penicillin
- 100μg/ml streptomycin
- 2.5μg/ml fungizone
- 10ng/ml TGF-β1
- 10^{-7}M dexamethasone
- 0.2mM ascorbic acid-2-phosphate
- 1mM Sodium Pyruvate
- 1:100 ITS+Premix

Bone Formation

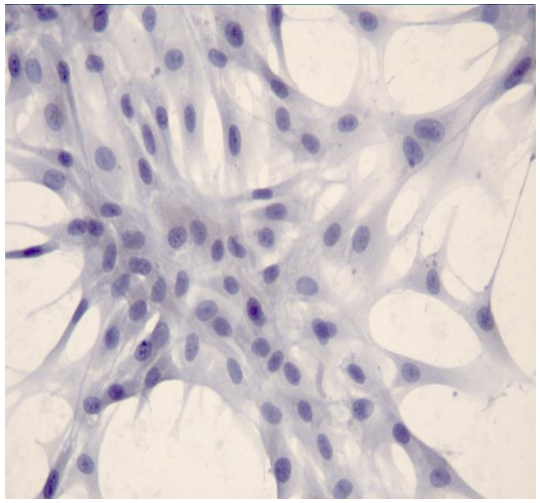
- Differentiation of MSCs to bone forming cells (osteoblasts)
- OBs synthese bone extracellular matrices
- Bone is formed and remodelled

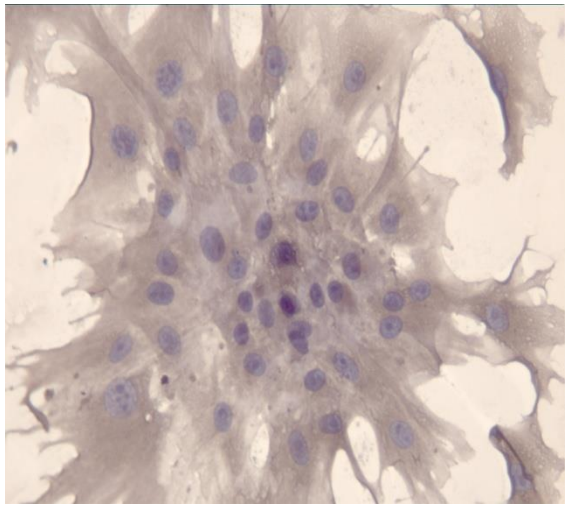


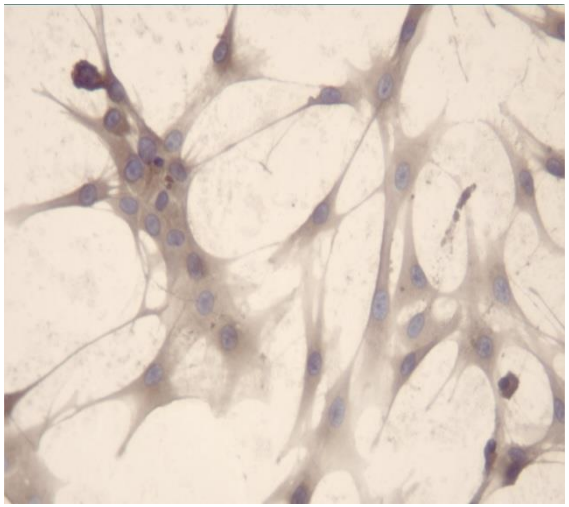
Markers for bone-forming cells (Osteoblasts)

- Molecular Markers
  - Alkaline phosphatase (ALP)
  - Type I collagen
  - Osteocalcin
  - Osteonectin
  - Bone Sialoprotein
- Cellular Markers
  - Calcium phosphate
    (detected by Alizarin Red stain)

BM





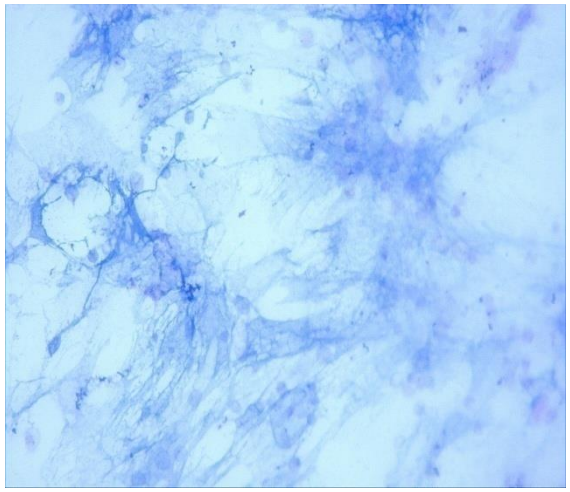


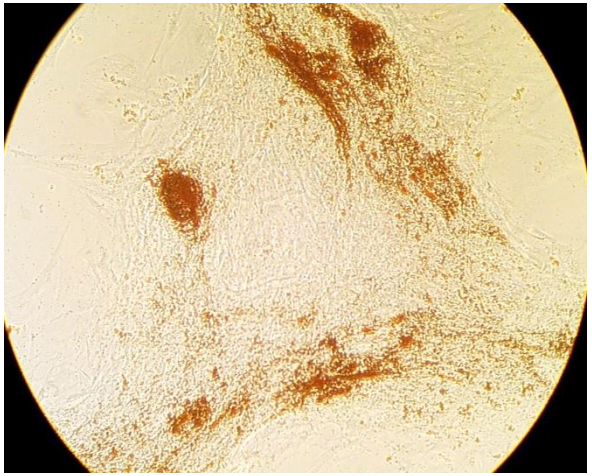
Negative control

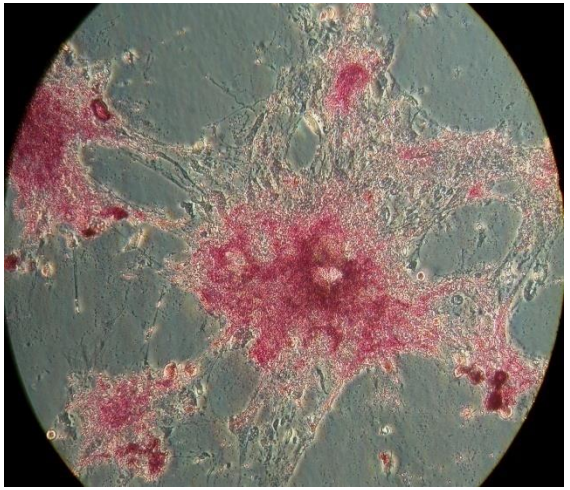
Type Ⅰ collagen

Osteocalcin

BM





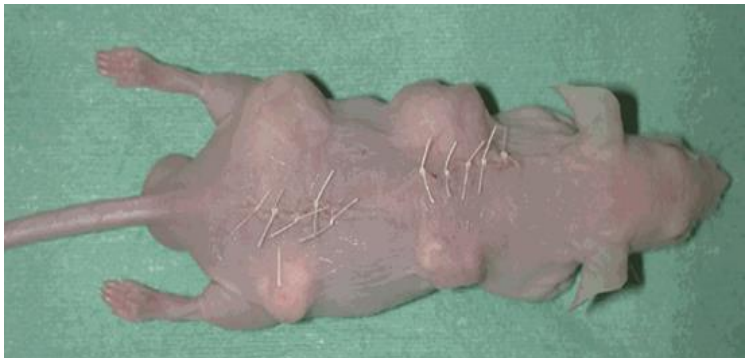


ALP

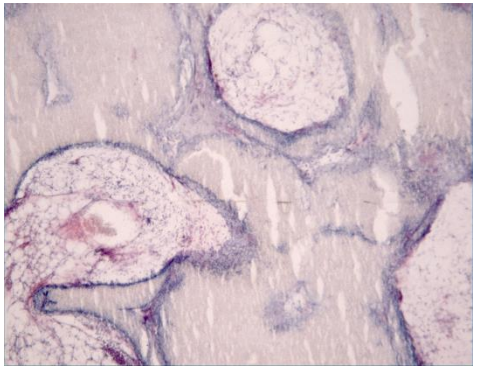
Von Kossa

Alizarin red

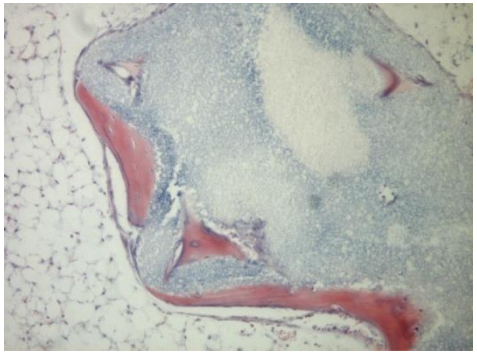
Differentiation Potential of MSCs- In Vivo Testing



In vivo Bone formation study

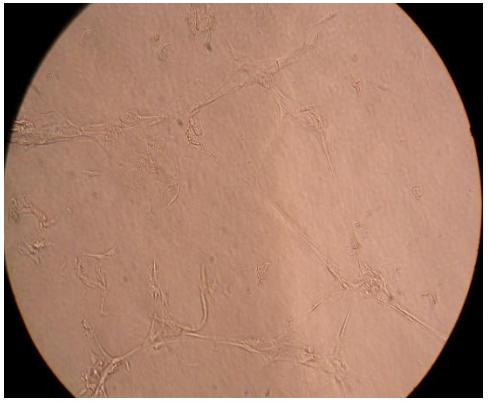


Non-cell CaP block 3month×50

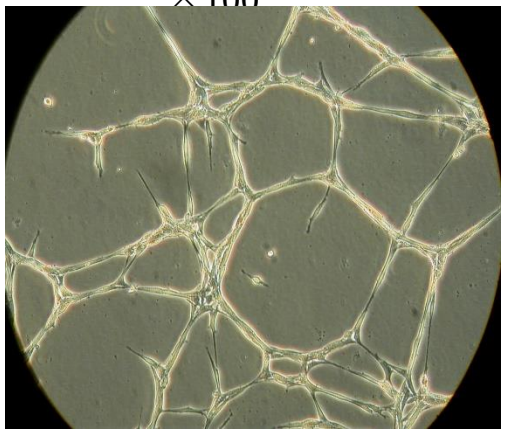


PBMSCsseeded CaP block 3month×50

In vitro angiogenesis

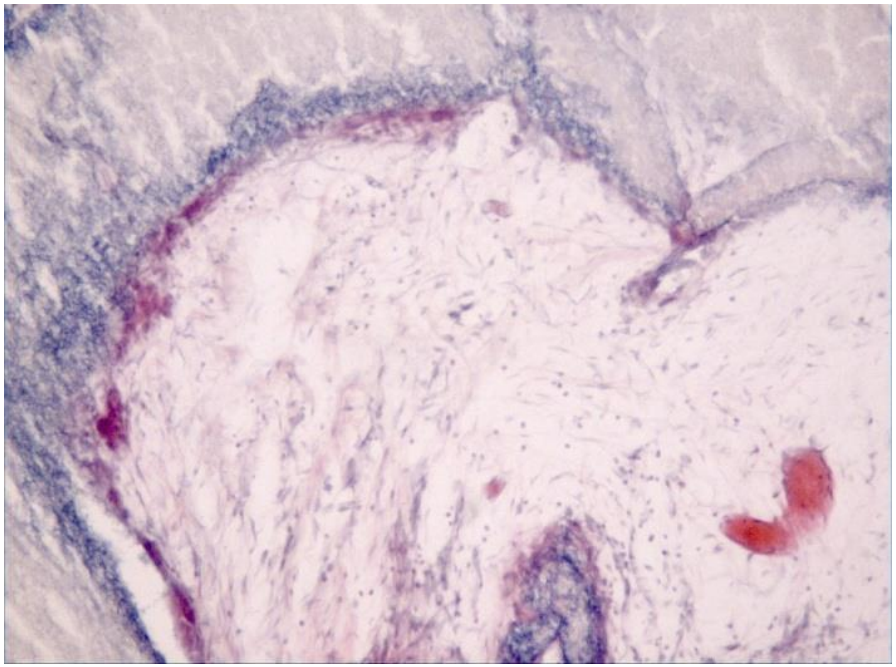


Matrigel 3D
culture 24h
×100

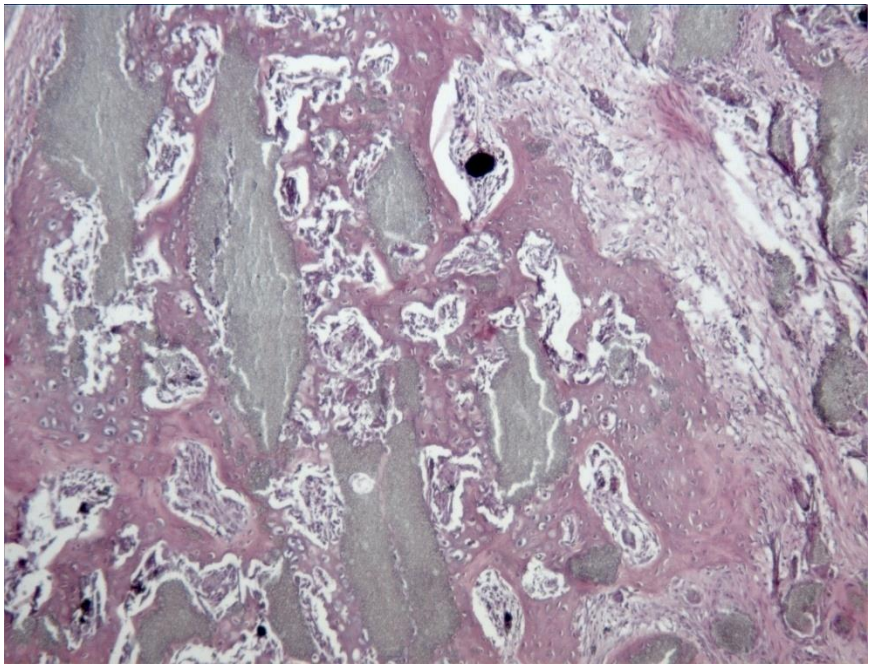


Long term 2D
culture 72h ×100

Histology: H&E Staining

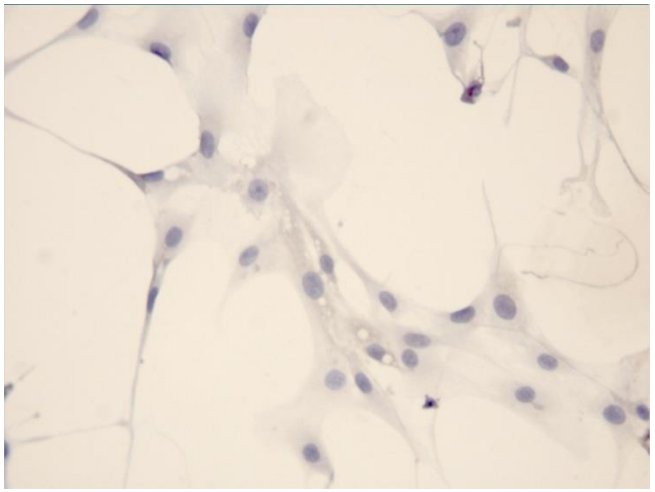


Biomaterials without BM cells

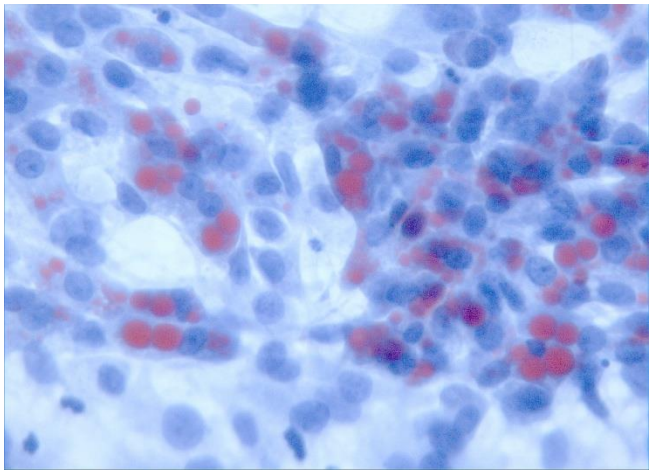


Biomaterials with BM cells

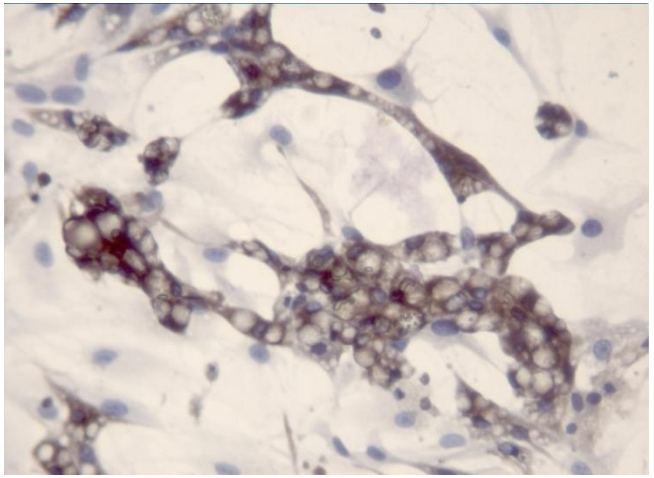
Adiopogenesis Induction Assay



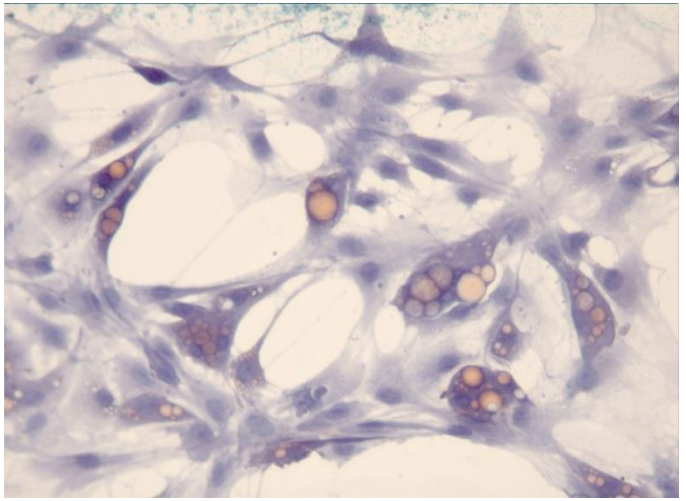
Negative control



Oil Red O

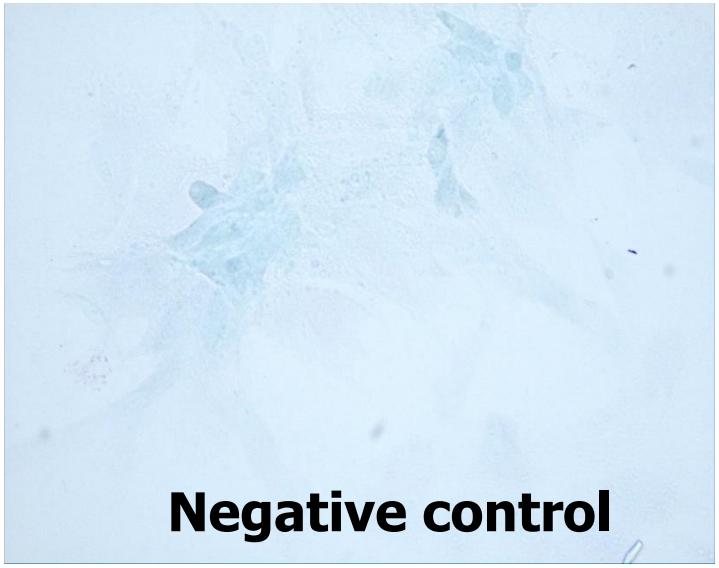


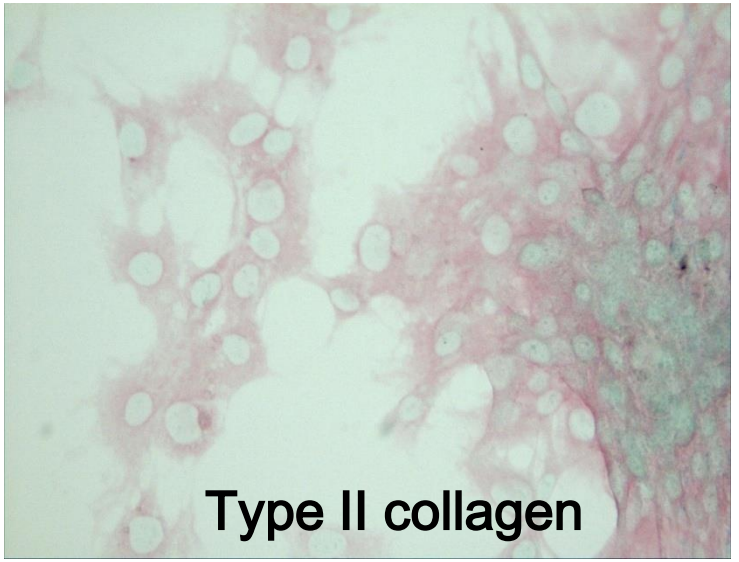
C/EBP α



Oil Red O

Chondrogenesis Induction Assay





Summary

- BM-MSCs are multiple potentials cells that can be induced into various cell lineages.

- BM-MSCs may be used as cell source for tissue-engineering applications.

Stem Cells - III

Stem Cells in Tissue Regeneration

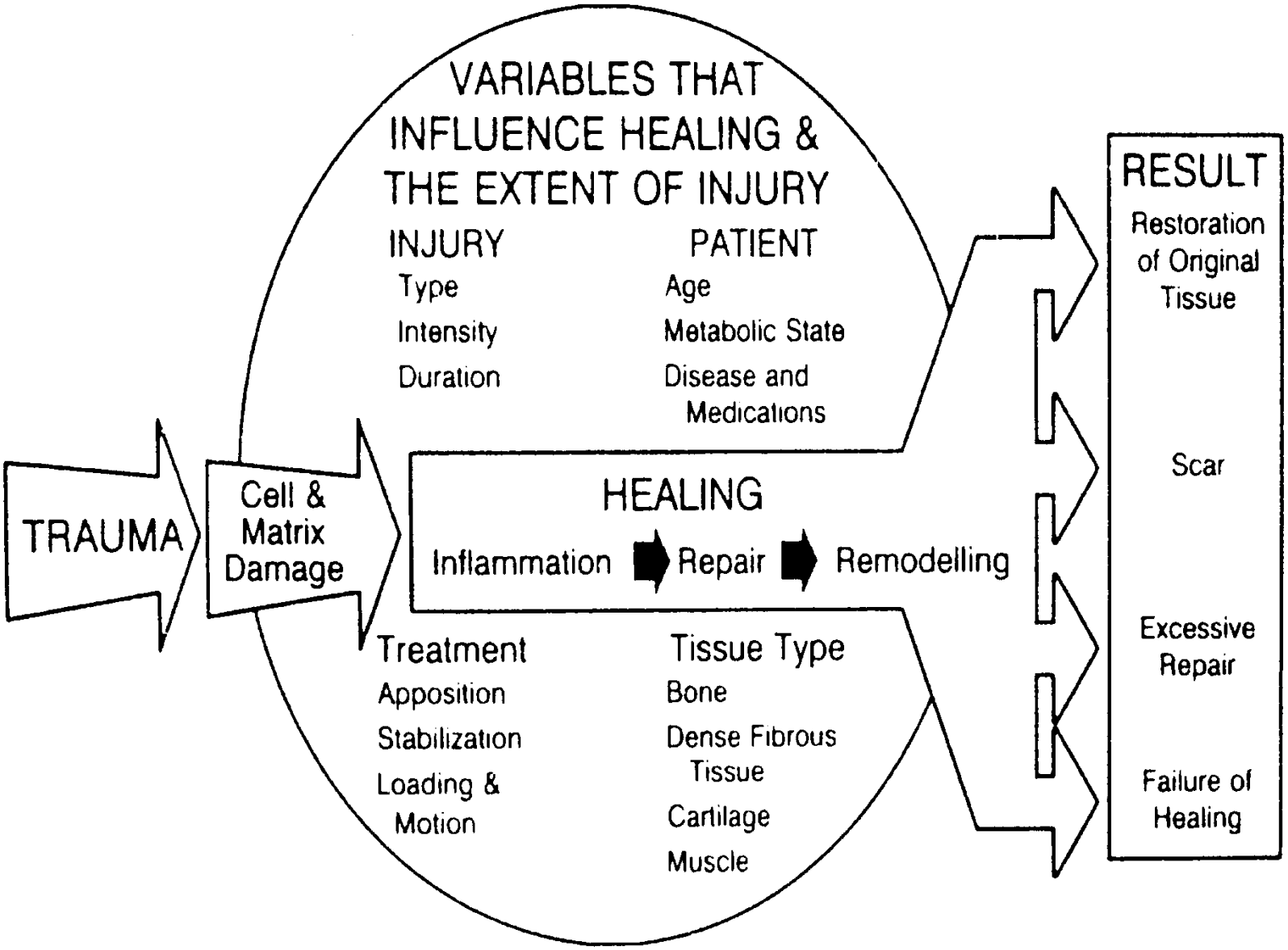
Prof. Gang Li, MBBS, DPhil (Oxon)

Stem Cell and Regeneration Program

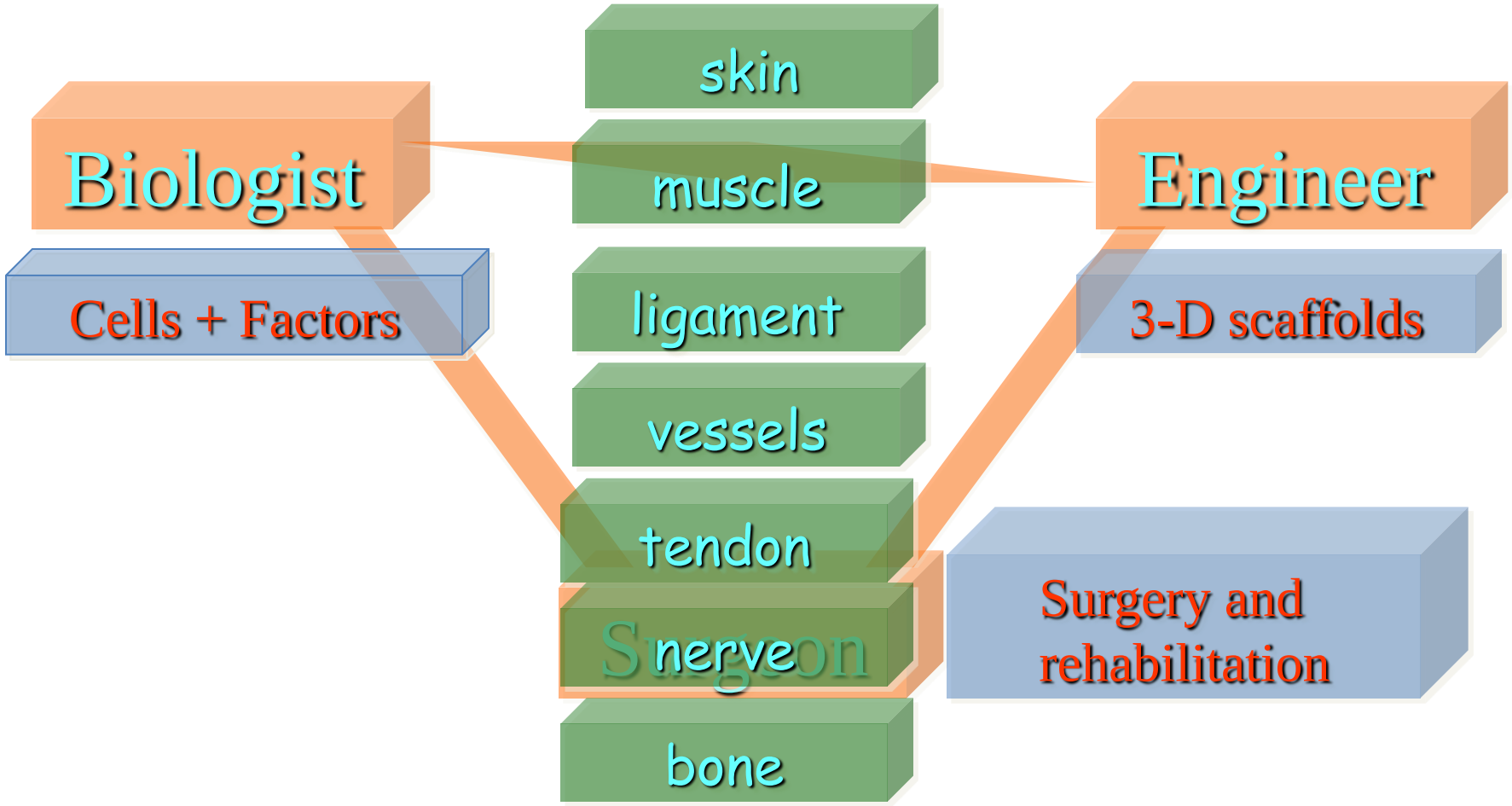
School of Biomedical Sciences

Dept of Orthopaedics &Traumatology

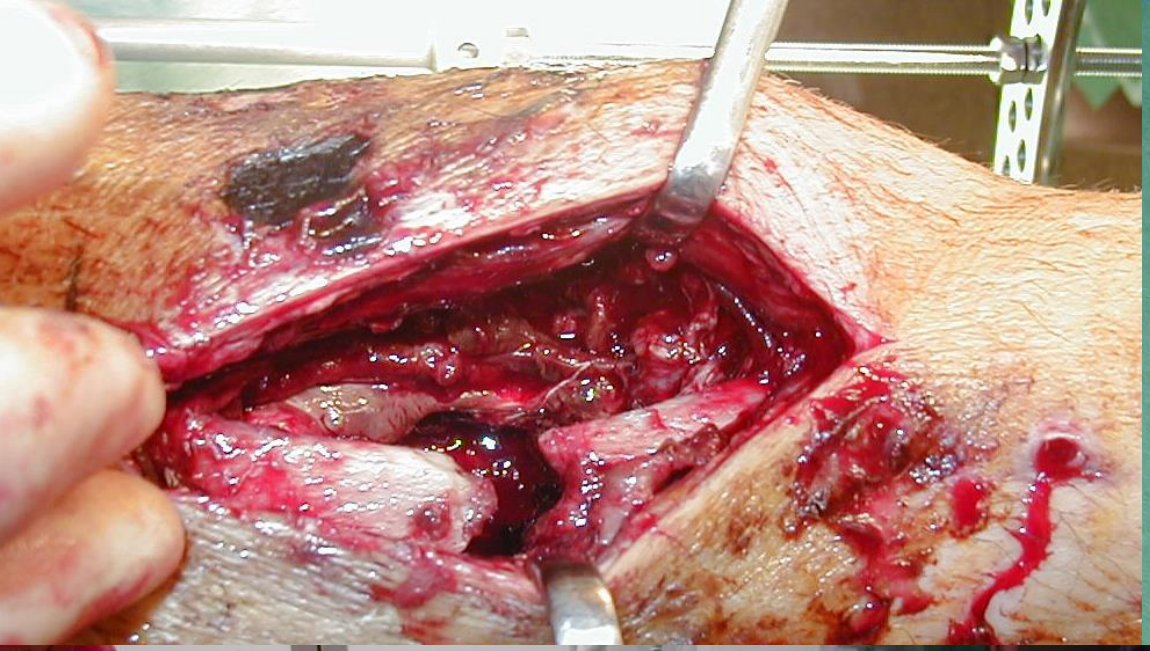
The Chinese University Hong Kong

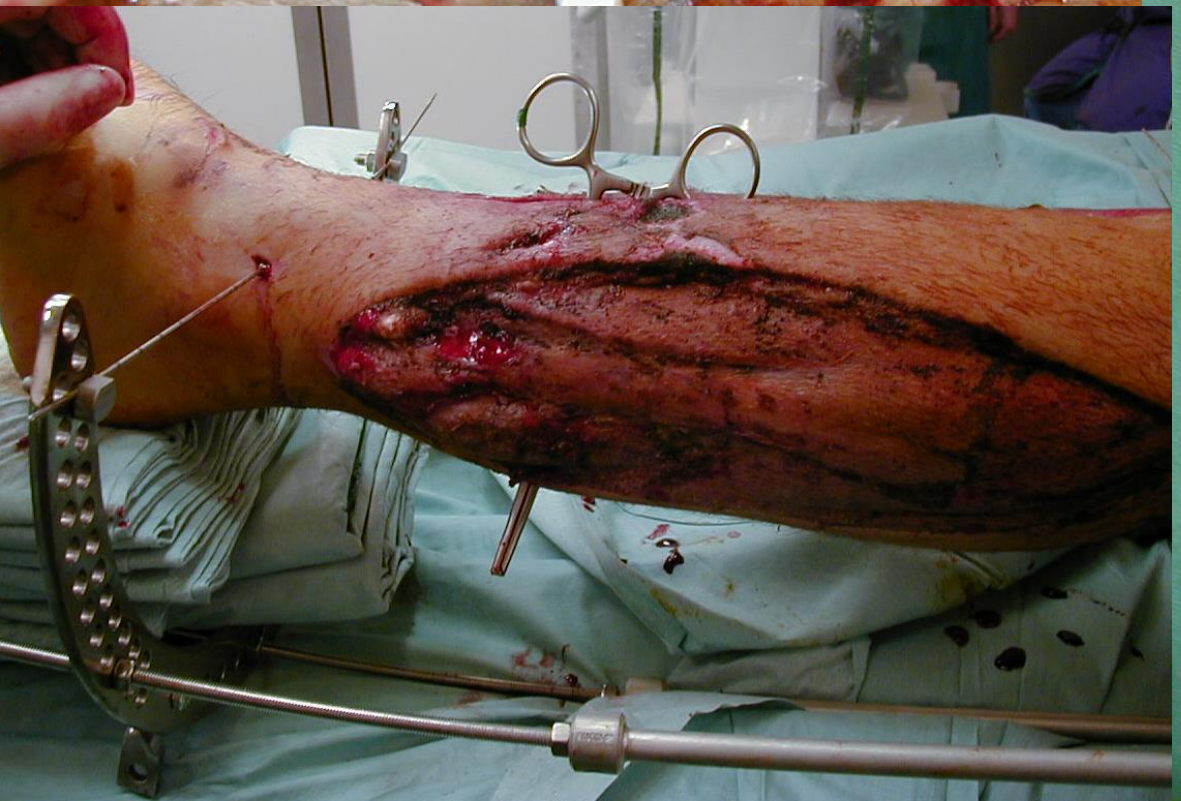

VARIABLES THAT INFLUENCE HEALING & THE EXTENT OF INJURY
INJURY
Type
Intensity
Duration
PATIENT
Age
Metabolic State
Disease and Medications
TRAUMA
Cell & Matrix Damage
HEALING
Inflammation
Repair
Remodelling
Treatment
Apposition
Stabilization
Loading & Motion
Tissue Type
Bone
Dense Fibrous Tissue
Cartilage
Muscle
RESULT
Restoration of Original Tissue
Scar
Excessive Repair
Failure of Healing

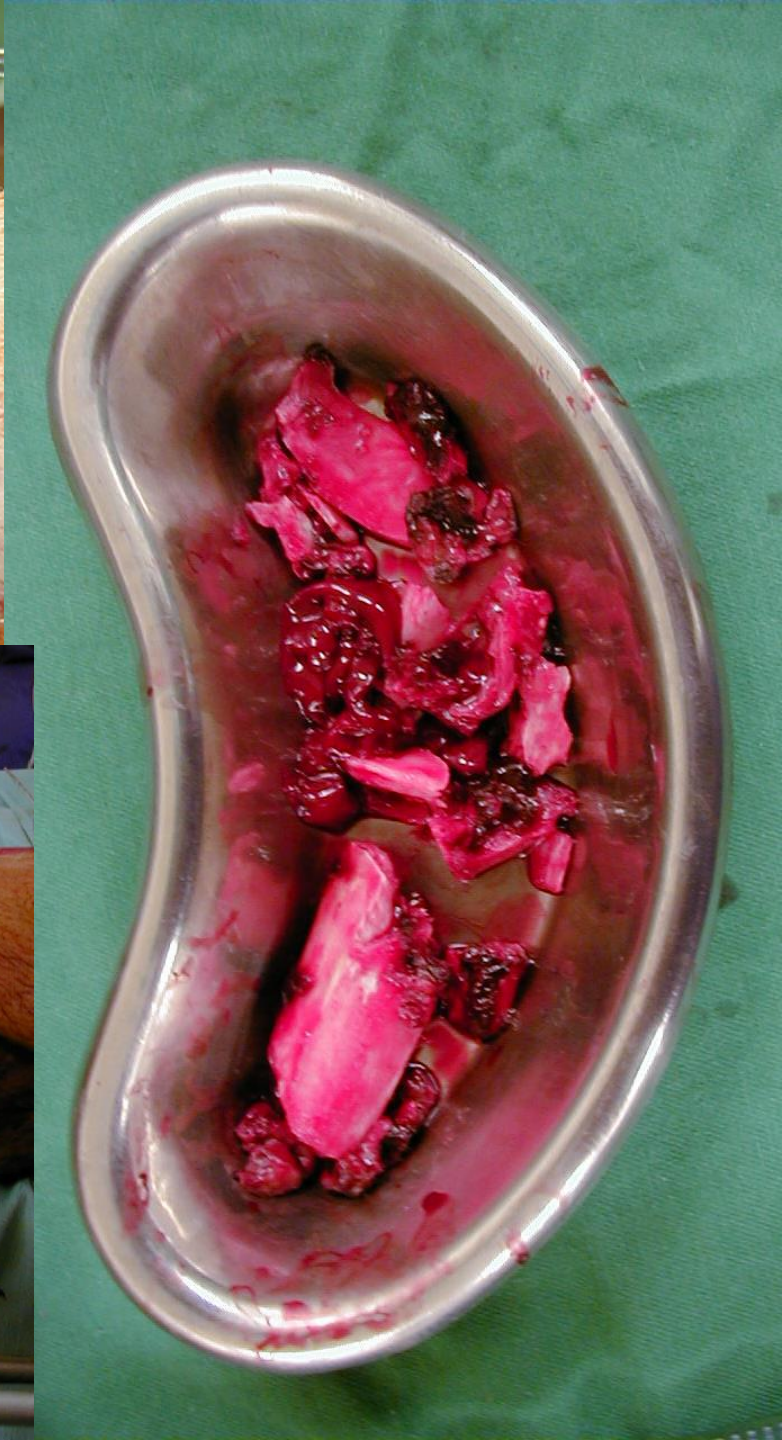
Tissue Engineering is involved with using cells and materials to form functional tissues or organs products




HELLO DOWN THERE..
EVERYTHING OK I TRUST?
Nicholson
9 SEPT 02

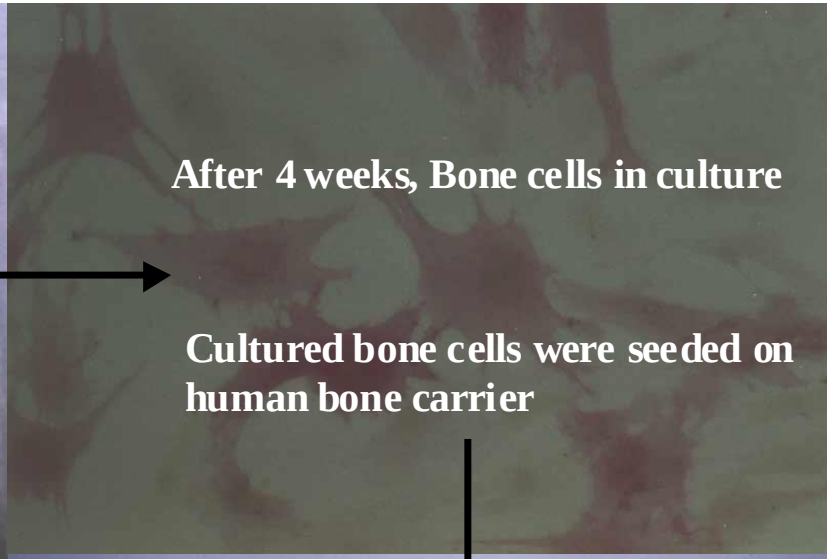






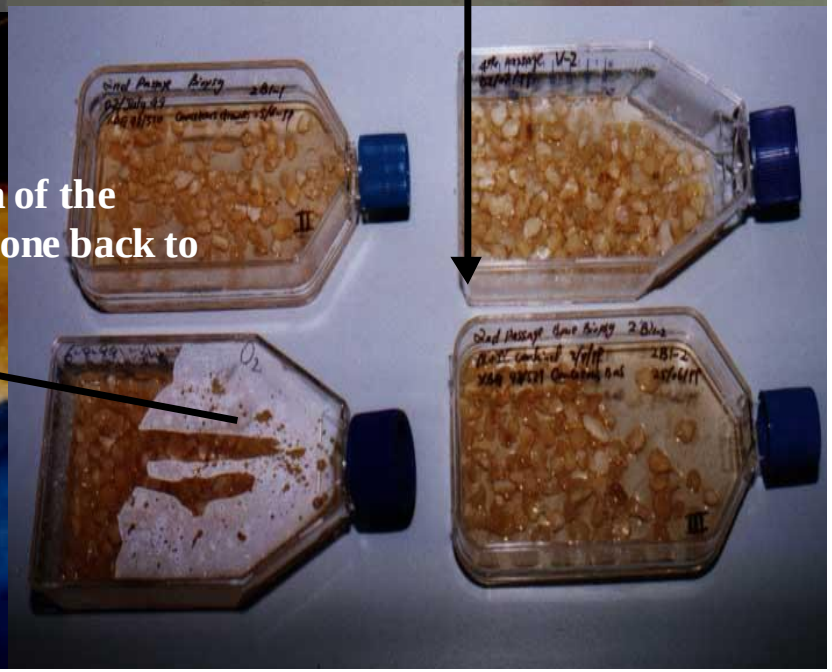
Clinical Trials of Using Autologous BM-MSCs for the treatment of bone defect

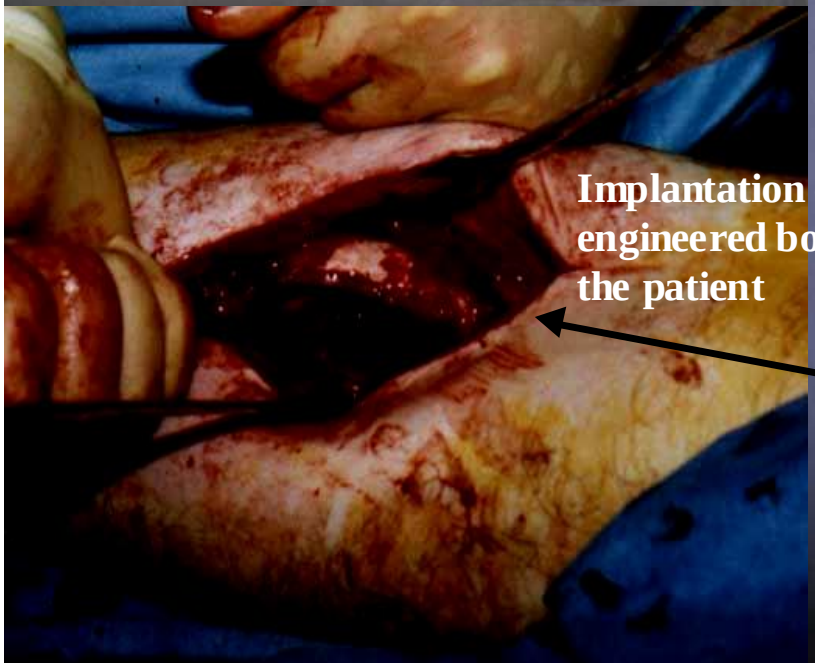
Bone biopsy in culture



After 4 weeks, Bone cells in culture

Cultured bone cells were seeded on human bone carrier



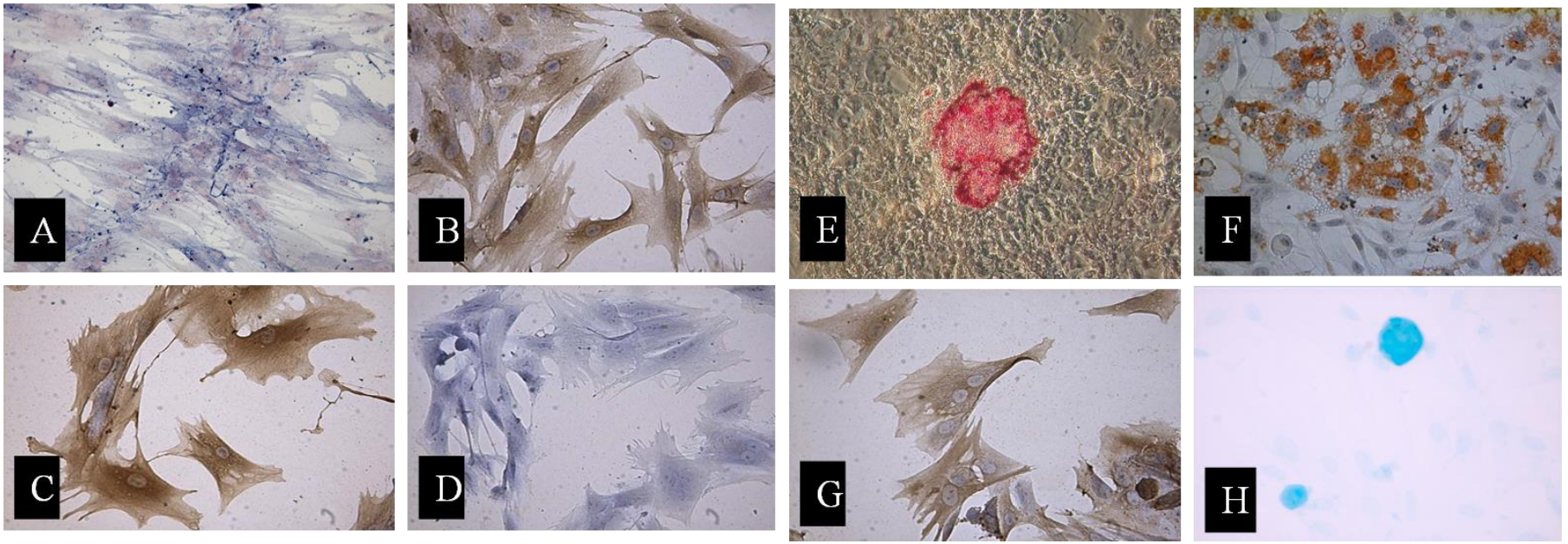


Implantation of the engineered bone back to the patient

Allogenic Peripheral Blood Derived Mesenchymal Stem Cells (MSCs) Enhance Bone Regeneration in Rabbit Ulna Critical-Sized Bone Defect Model

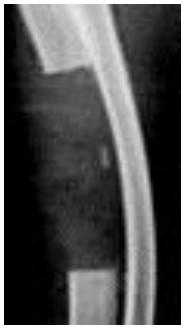
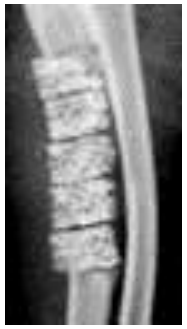
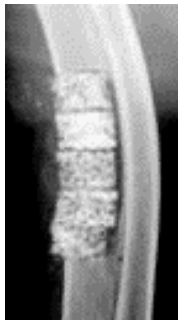
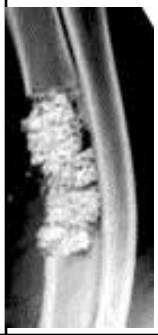
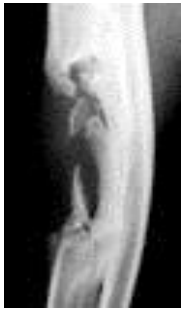
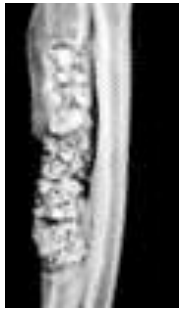
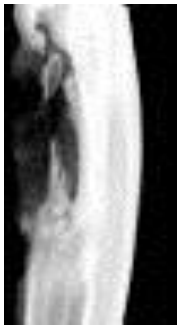
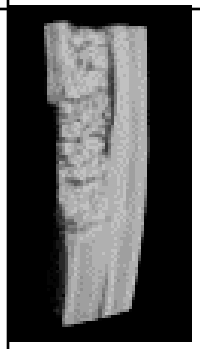
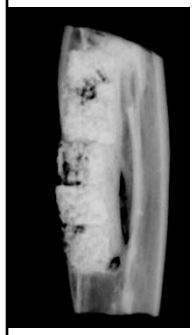
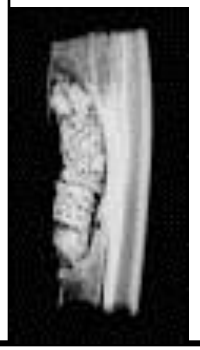
Chao Wan, Qiling He, Gang Li

Musculoskeletal Education and Research Unit, Centre for Cancer Research and Cell Biology,
School of Biomedical Sciences, Queen's University Belfast, Musgrave Park Hospital, Belfast, BT9 7JB, United Kingdom



Journal of Orthopaedic Research; 2006; 24(4):610-8.

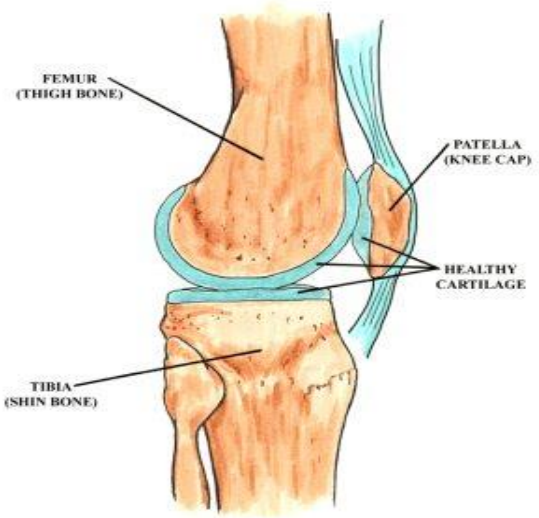
- Rabbit
- PBMSCs
- Repair cortical-sized bone defect

Groups	Empty Control	Skelite Alone	PBMSC Skelite	BMMSC Skelite	PBMNC Skelite
Day 0					
Week 8					
Week 12					

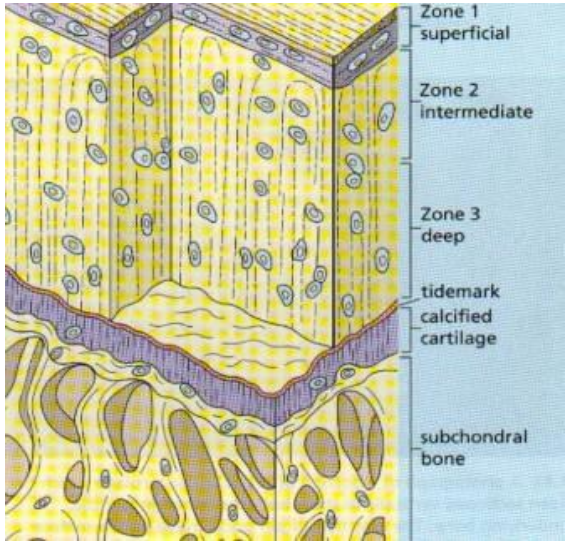
Wan C, He Q, Li G. Allogenic peripheral blood derived mesenchymal stem Cells (MSCs) enhance bone regeneration in Rabbit ulna critical sized bone defect model.

Journal of Orthopaedic Research; 2006; 24(4):610-8.

Articular cartilage



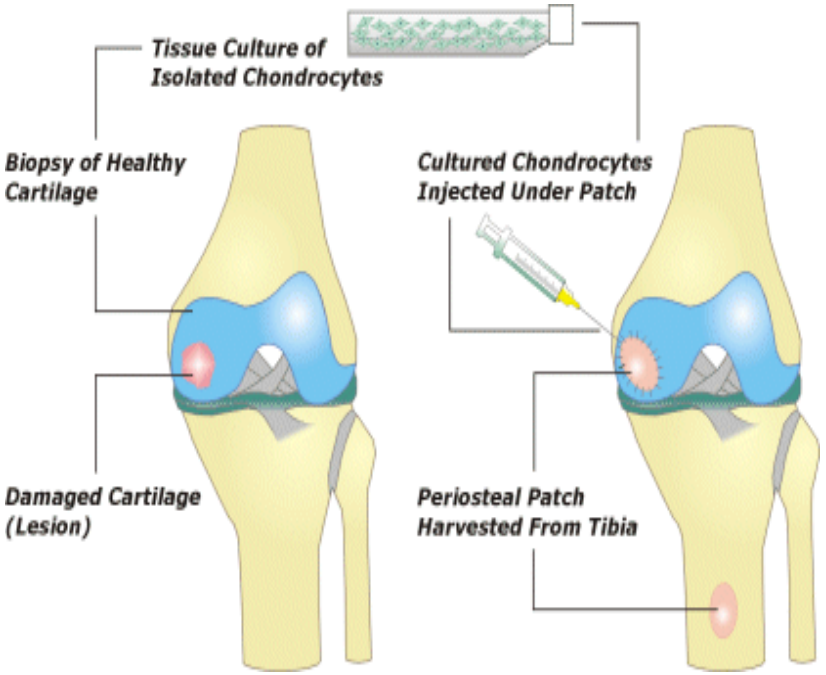
- Glassy tissue (hyaline cartilage)
- Importance
  - provide frictionless movement
  - Absorb mechanic shocks
  - distribute loads
- Special structure
  - only chondrocytes
    - Sparsely distributed
    - embedded in dense ECM
    - limited proliferation
  - avascular aneural alymphatic



Cartilage injury & joint replacement

- Cartilage injuries
  - very common in daily life
  - 15% population in U.K.
  - secondary osteoarthritis and disablement
- Advanced stage--joint replacement
  - over 70,000 cases /year
  - over £5 billion/year
  - great economic burdens for society and families

Cartilage Tissue engineering

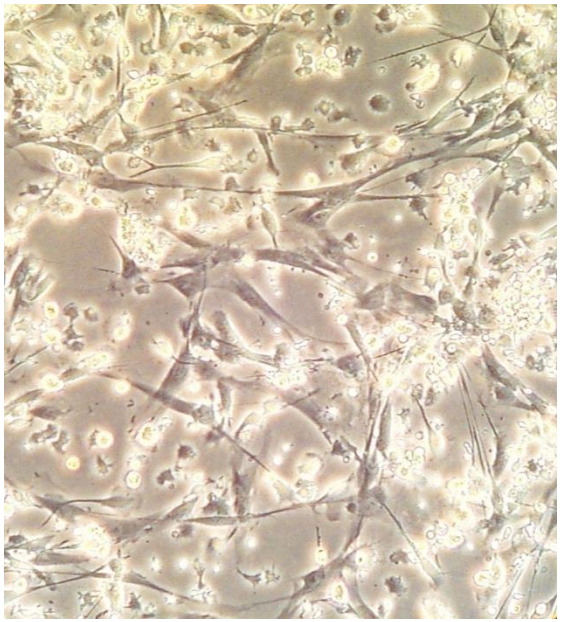


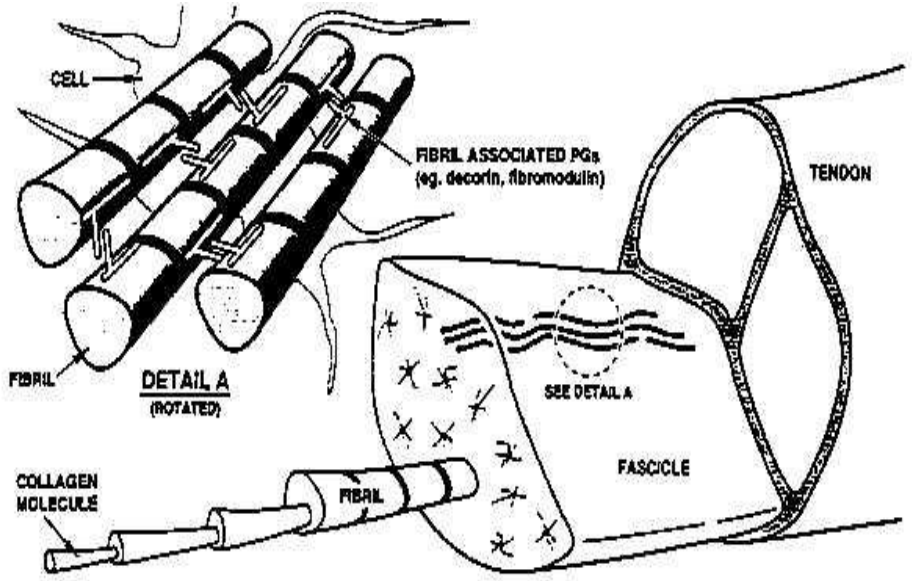
- Interdisciplinary science
  - engineering
  - life sciences
- Three basic elements
  - Cells
  - Scaffolds
  - Bioactive molecules

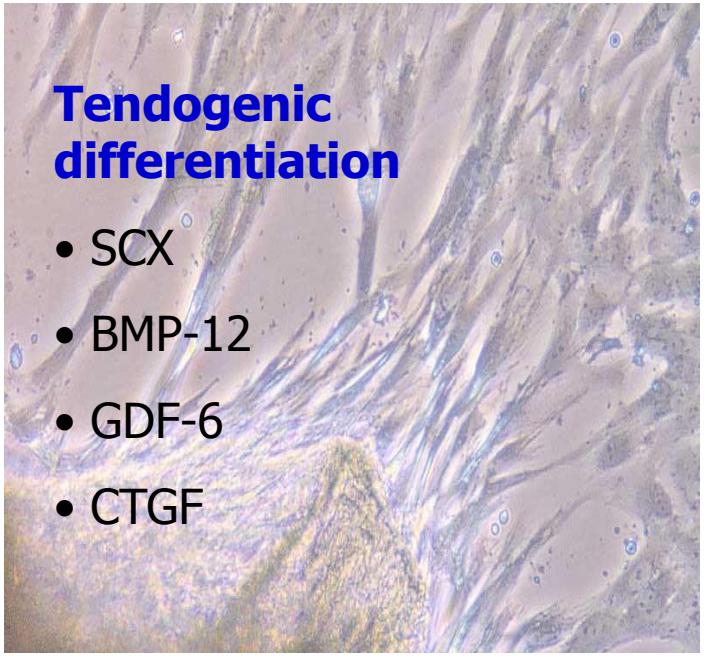
ACT (autogenous chondrocyte transplantation) more than 12,000 patients worldwide

Tendon differentiation

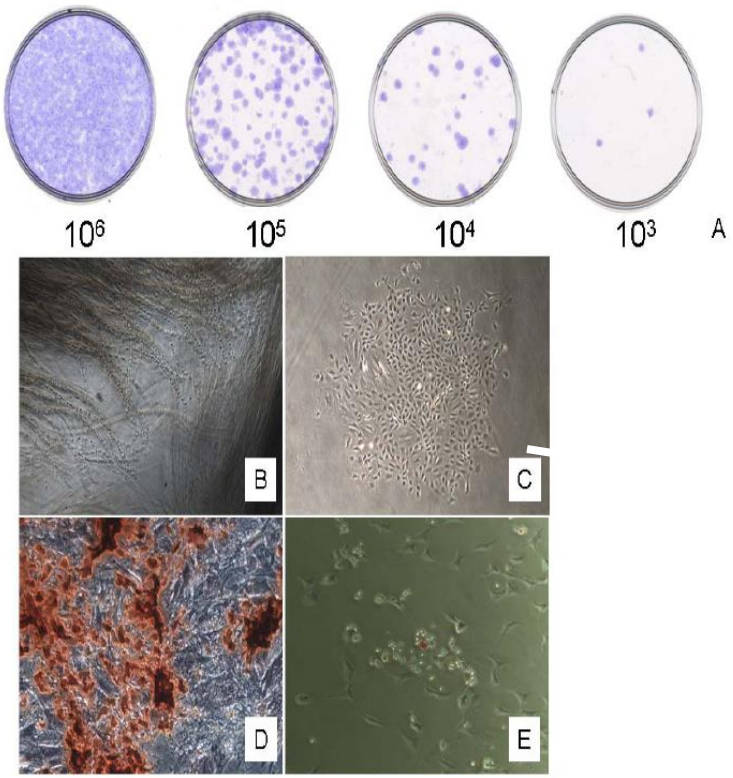
- Tendogenic differentiation
- Specific factors and culture condition
- Standard techniques

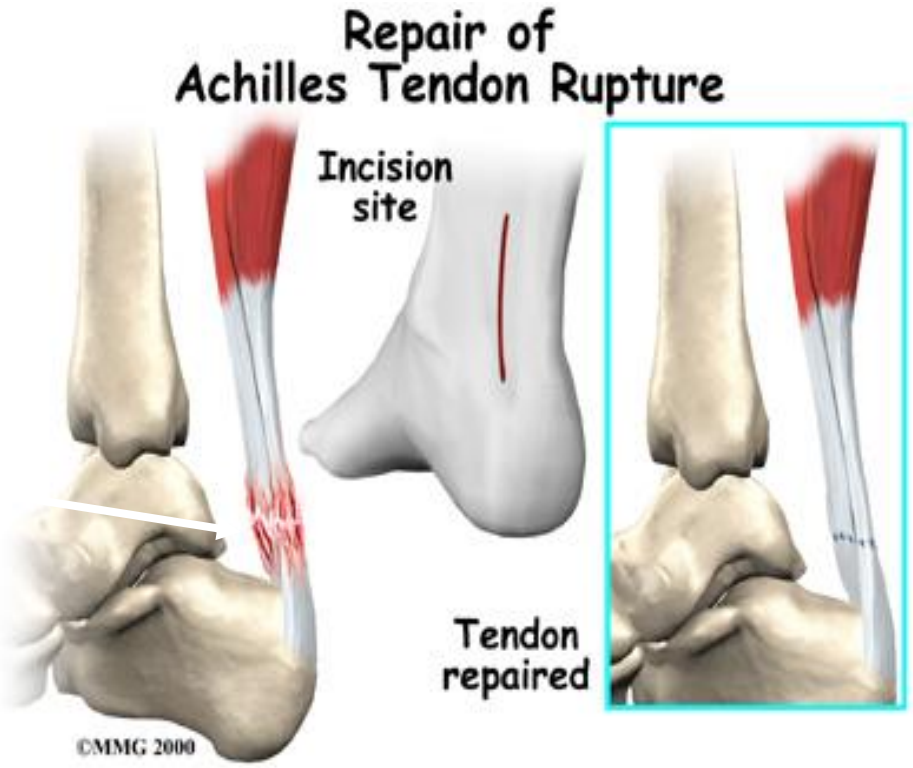






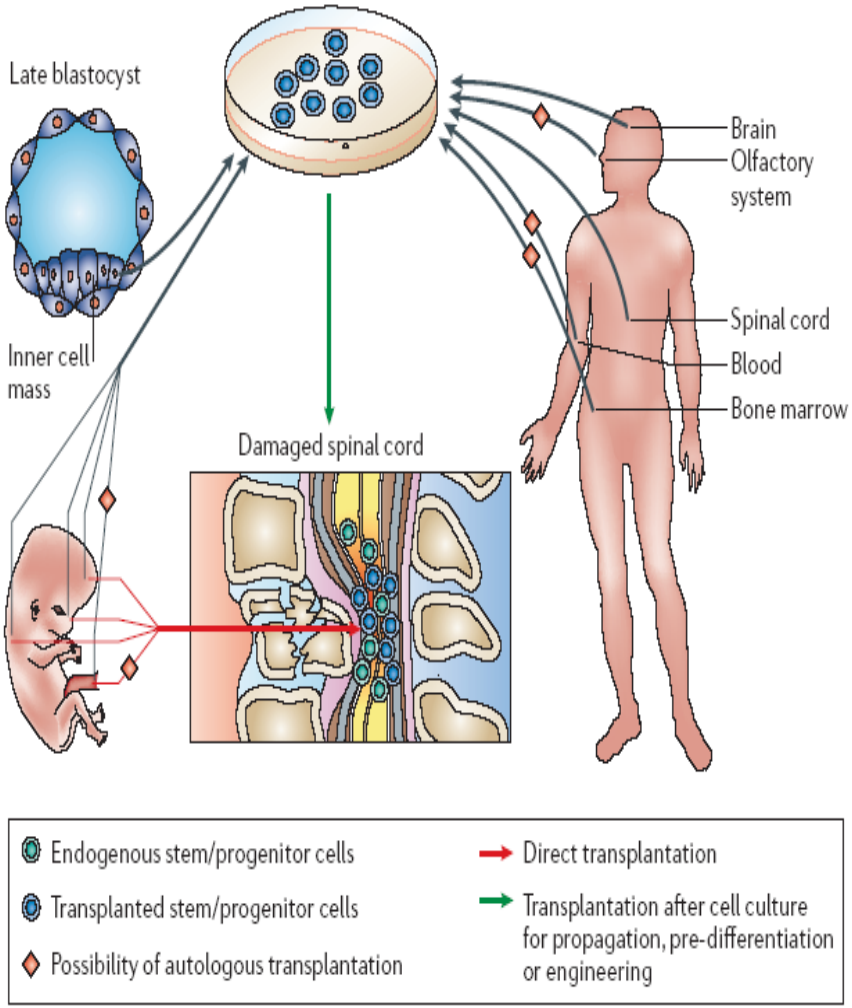
Tendon stem cell differentiation





Tendon derived stem cells can also differentiate into bone, cartilage, fibroblast and other cell lineages, they are multi-potent stem cells, responsible for tendon repair.

Cellular Therapeutic Interventions for SCI



NATURE REVIEWS NEUROSCIENCE, 2006: 7:628-43.

Transplantation of: -

- **Peripheral nerve**
- **Schwann cells**
- **Olfactory nervous system cells**
- **Embryonic CNS tissue**
- **Embryonic stem cells**
- **Adult stem/progenitor cells**
- **Engineered stem cells**
- **Activated macrophages**

Mainly in animal models

Small numbers of cases

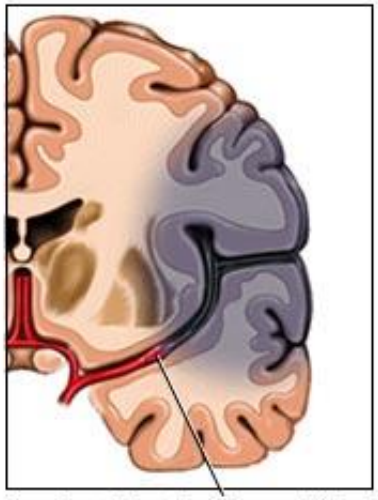
Limited success

None of them conclusive

Lack of controlled trails

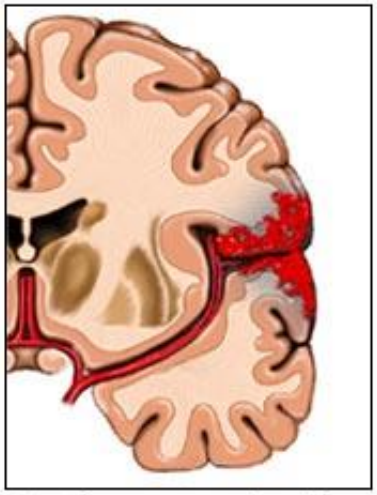
Stroke / Cell Therapy

Ischemic stroke

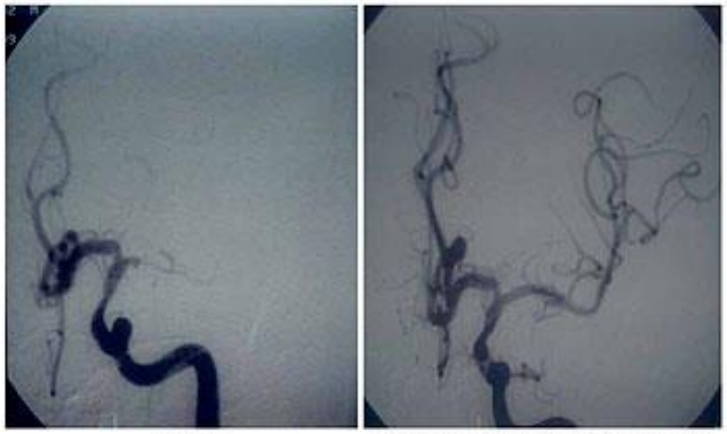


A clot blocks blood flow to an area of the brain

Hemorrhagic stroke



Bleeding occurs inside or around brain tissue



Before

After Intra-arterial Thrombolysis

Minimizing the damages

- **Stop bleeding**
- **Unblock the vessels**

Maximizing the changes of functional recovery

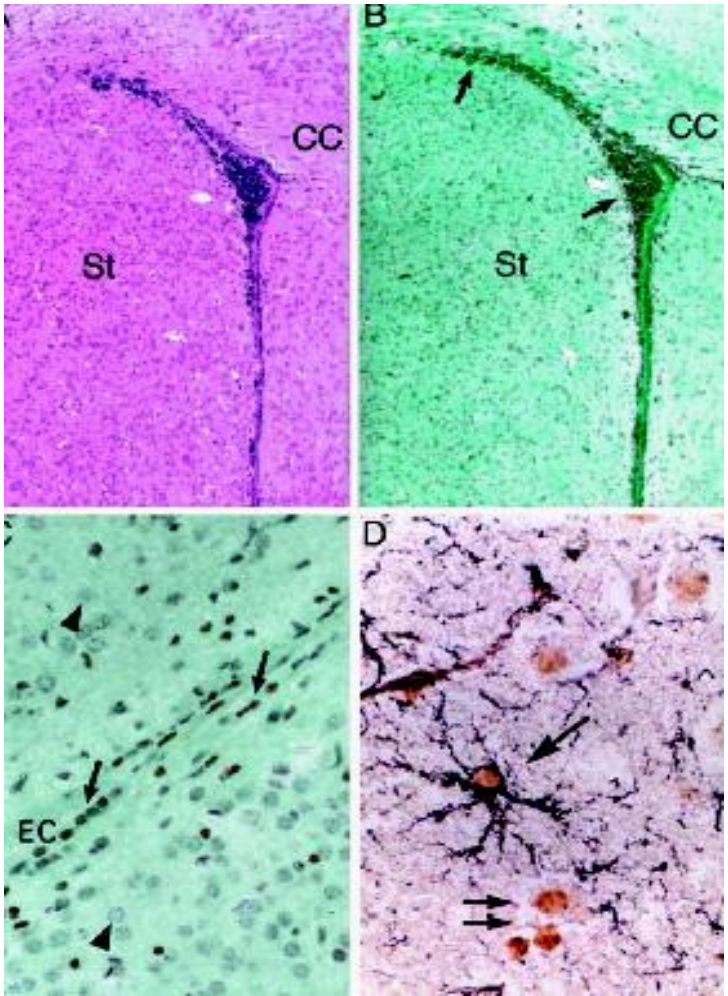
- **Control inflammation**
- **Restore blood supply**
- **Active physiotherapy**

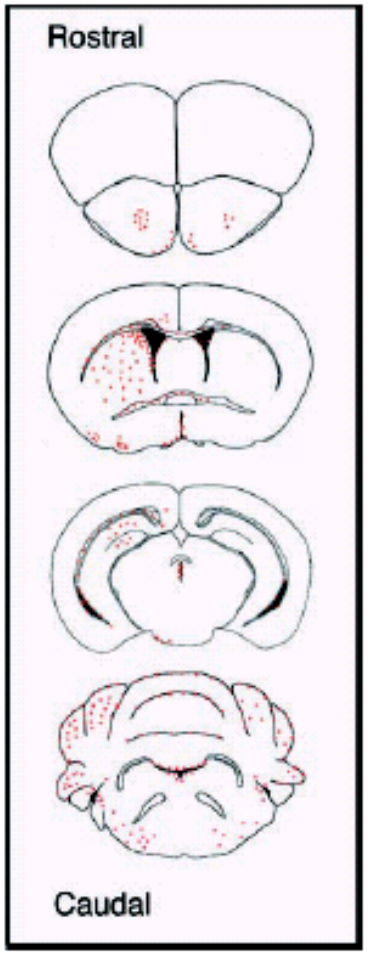
Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 10711–10716, September 1999
Cell Biology

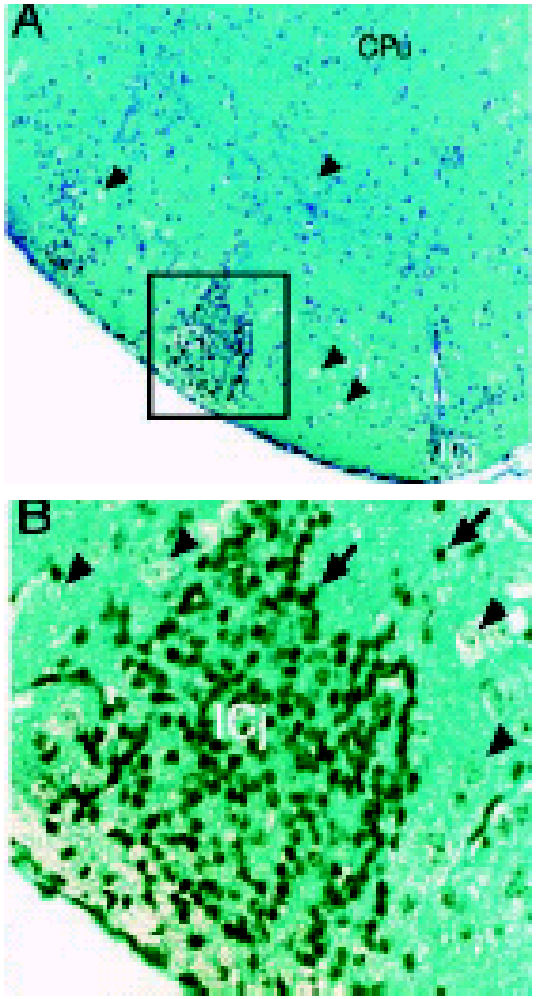
Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains

GENE C. KOPEN, DARWIN J. PROCKOP, AND DONALD G. PHINNEY*

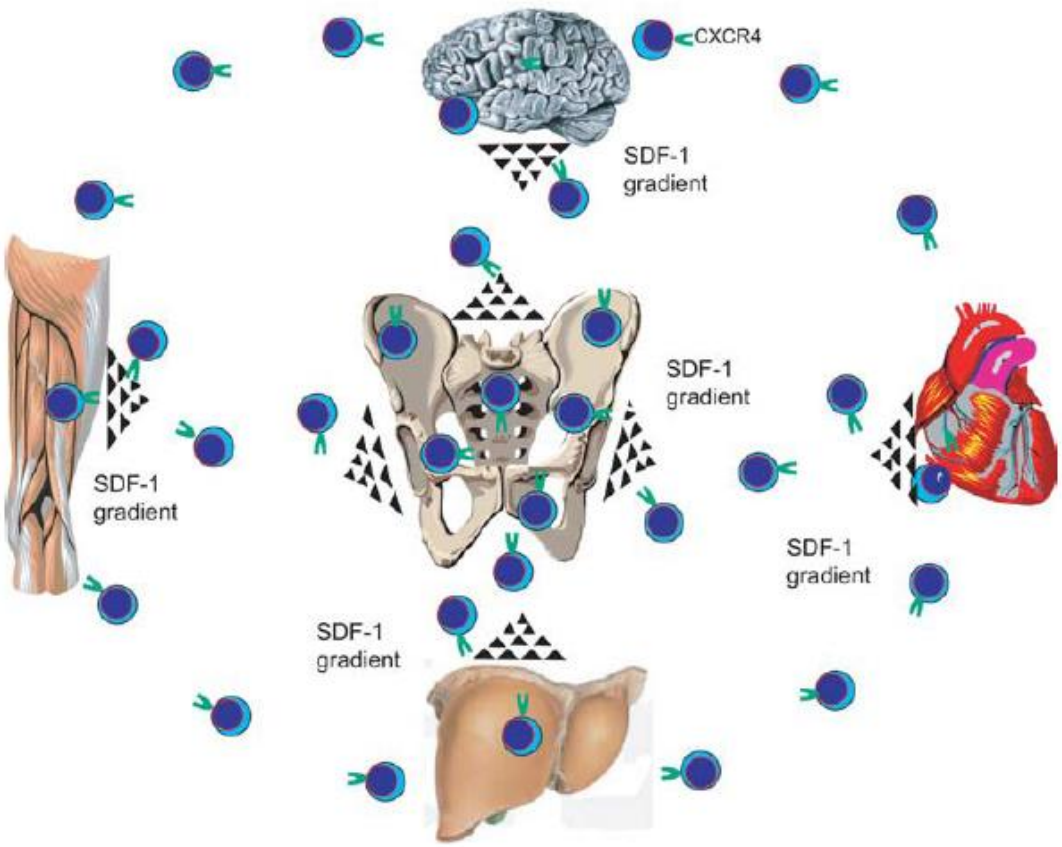
Center for Gene Therapy, MCP Hahnemann University, 245 North 15th Street, Philadelphia, PA 19102-1192







MSCs Home to Injury Sites



MSCs home to a variety of tissues, particularly after tissue injury and ischemia.

Miyahara Y, Nagaya N, Kataoka M,et al . Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med. 2006 Apr;12(4):459-65.

Carvalho KA, Guarita-Souza LC, Hansen P,et al. Cell Transplantation After The Coculture of Skeletal Myoblasts and Mesenchymal Stem Cells in the Regeneration of the Myocardium Scar: An Experimental Study in Rats. Transplant Proc. 2006 Jun;38(5):1596-1602.

Gnecchi M, He H, Noiseux N,et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J. 2006 Apr;20(6):661-9.

Kraitchman DL, Tatsumi M, Gilson WD, et al. Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction. Circulation. 2005 Sep 6;112(10):1451-61.

ELSEVIER

Journal of Orthopaedic Research 23 (2005) 1013–1021

Journal of Orthopaedic Research

www.elsevier.com/locate/orthres

Systemic recruitment of osteoblastic cells in fracture healing

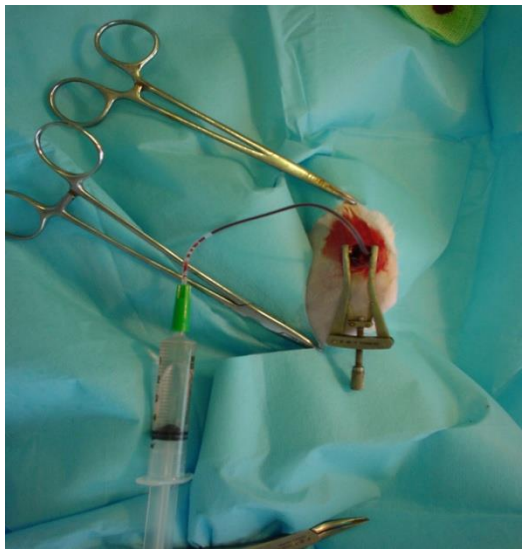
Denise Shirley [a], David Marsh [a], Grant Jordan [a], Stephen McQuaid [b], Gang Li [a,*]

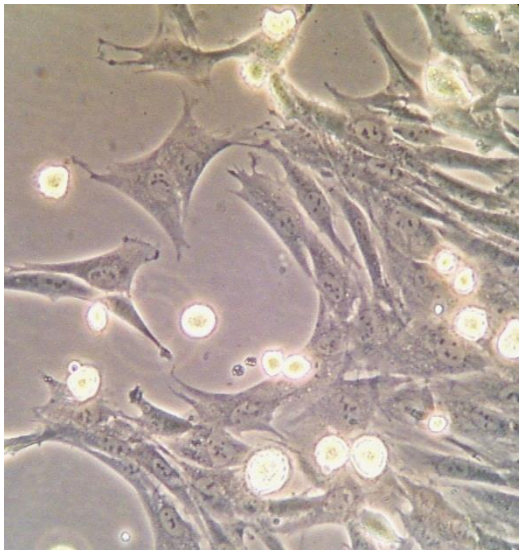
[a] *Department of Trauma and Orthopaedic Surgery, School of Medicine, Queen's University Belfast, Musgrave Park Hospital, Belfast BT9 7JB, UK*

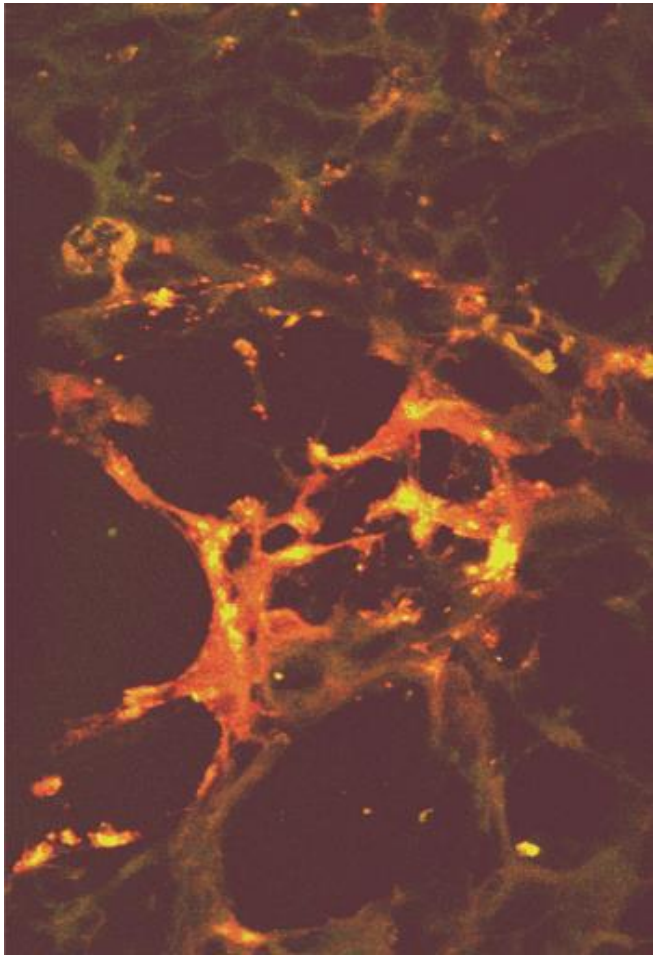
[b] *Department of Pathology, Royal Victoria Hospital, Belfast BT12 6BJ, UK*

Accepted 28 January 2005

MSCs home to fracture sites via circulation

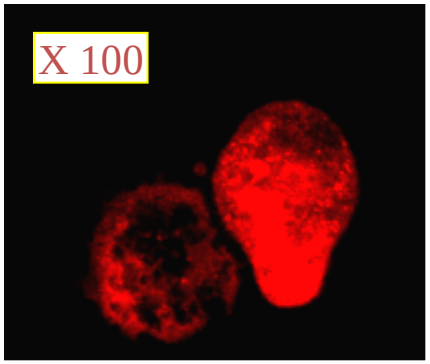






Bone marrow harvested

Rabbit bone marrow
MSCs culture



Shirley D, et al, Journal of Orthopaedic Research, 2005, 23 (5): 1013-21.

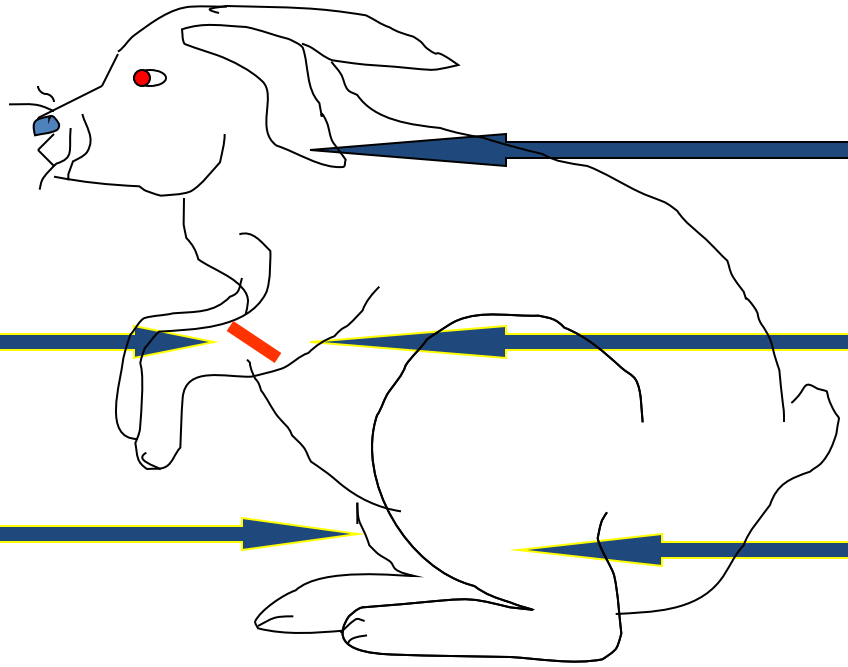
Re-implantation

48 hours post Fx

In each group some animals

were sacrificed at 3 & 12 weeks

Culture 3 weeks



Control E

Ear vein B

Ulnar defect

Fracture site A

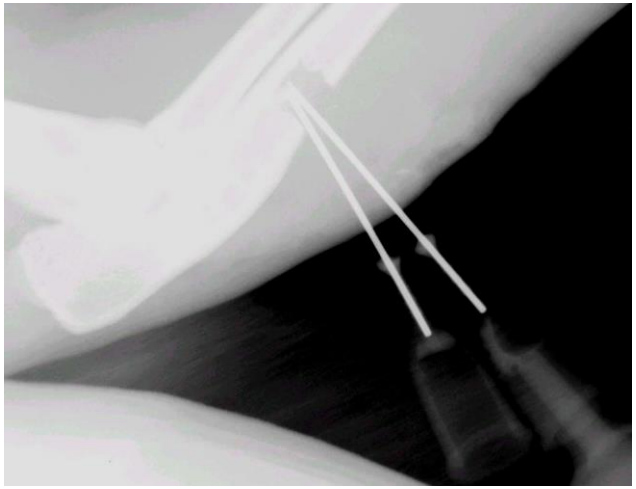
BM harvest

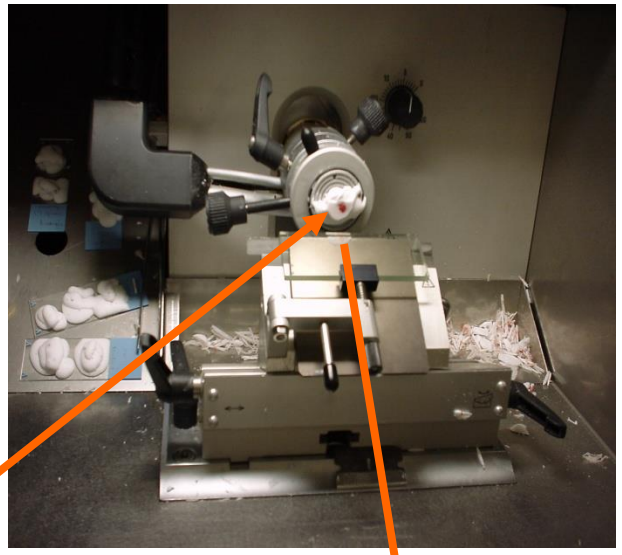
Remote BM site C

Shirley D, et al, Journal of Orthopaedic Research, 2005, 23 (5): 1013-21.

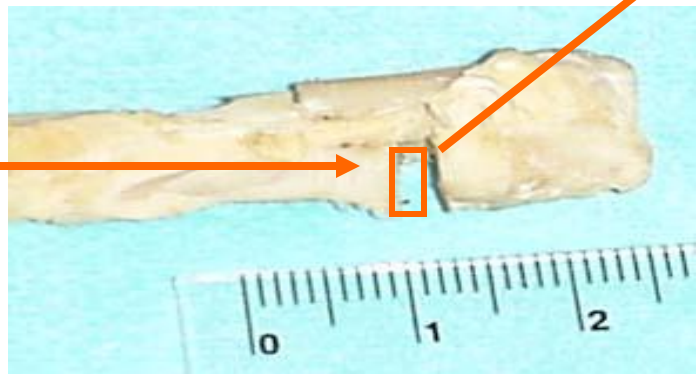
The tissues retrieved for frozen section – (5μm)

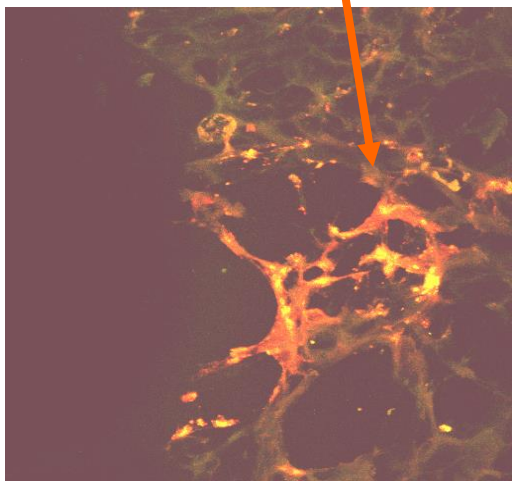
Animals were sacrificed at 3 and 12 weeks after cell implantation





Gap tissue



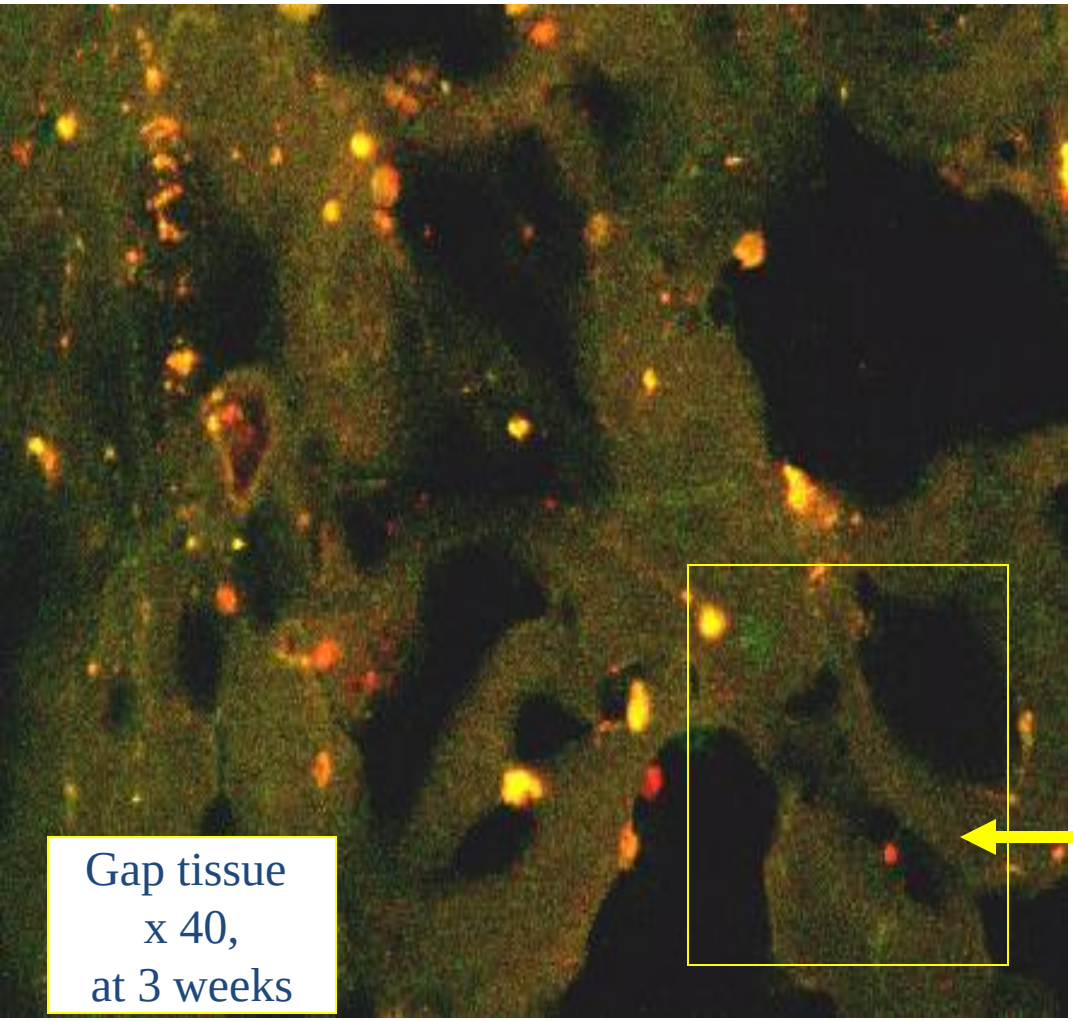


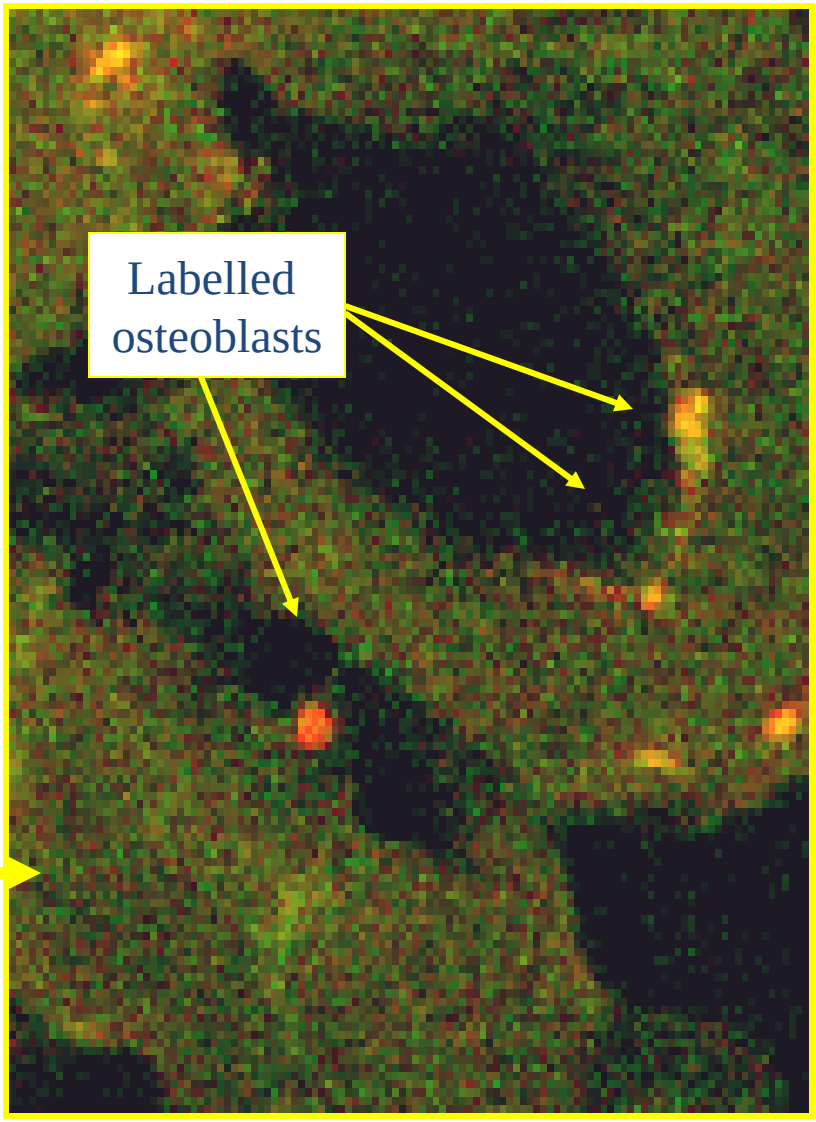
– Liver, lung, kidney, and spleen,

– Also cytospins of BM and blood

(representative samples only)

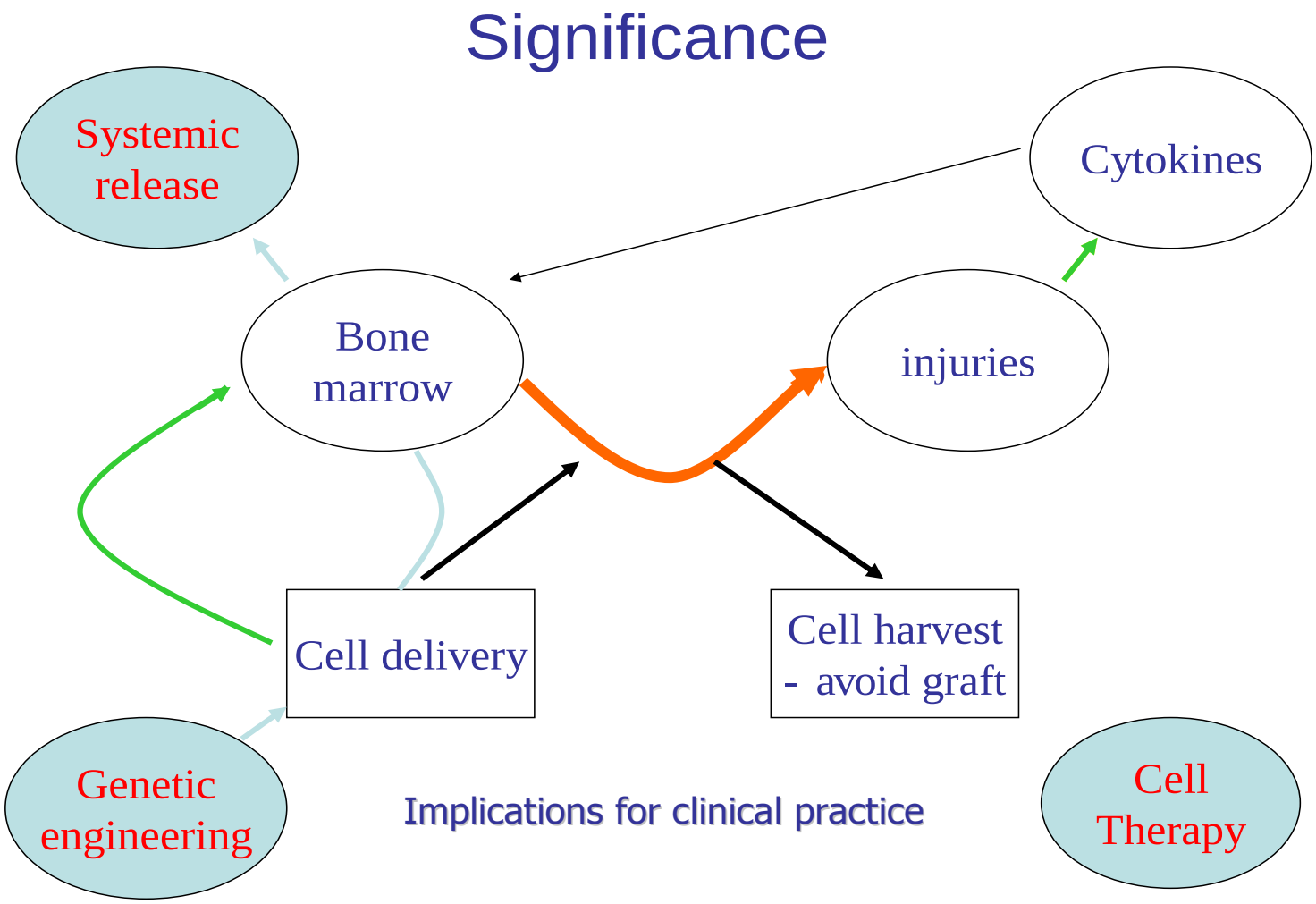
Labelled cells from remote marrow identified in fracture gap (Group C)





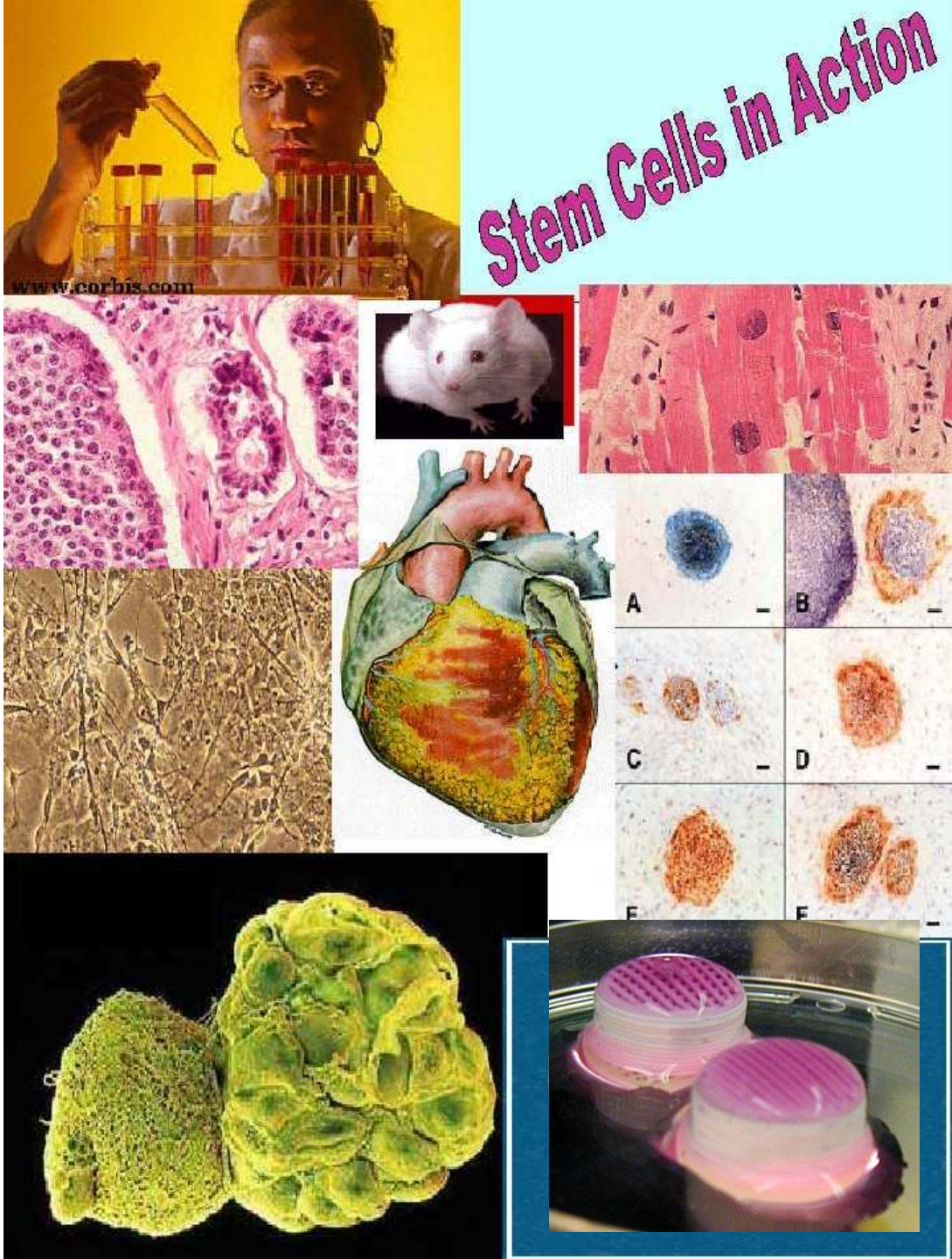
Shirley D, et al, JOR, 2005, 23 (5): 1013-21.

- **Some osteoblasts in fracture repair come from remote bone marrow sites via systemic circulation.**
- **They are actively recruited and homed to fracture sites.**



Stem cell therapy application

- Autoimmune Diseases
- Cerebral Palsy
- Critical Limb Ischemia
- Degenerative Joint Disease
- Diabetes Type 2
- Heart Failure
- Multiple Sclerosis
- Osteoarthritis
- Rheumatoid Arthritis
- Spinal Injury



Summary

- **BM-MSCs, umbilical cord blood MSCs and tissue specific MSCs are all good sources.**
- **Cell expansion techniques to allow rapid proliferation or controlled differentiation.**
- **Off shelf, ready to use cell products are available.**
- **Intelligent biomaterials for special needs.**
- **Novel techniques of growth factors slow release and cell preparations reduce costs and enhance tissue repair.**
- **Cell based gene therapy for tissue regeneration is promosing.**


Thank you！ 谢谢！
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