Promoting Fracture Healing Through Systemic or Local Administration of Allogeneic Mesenchymal Stem Cells

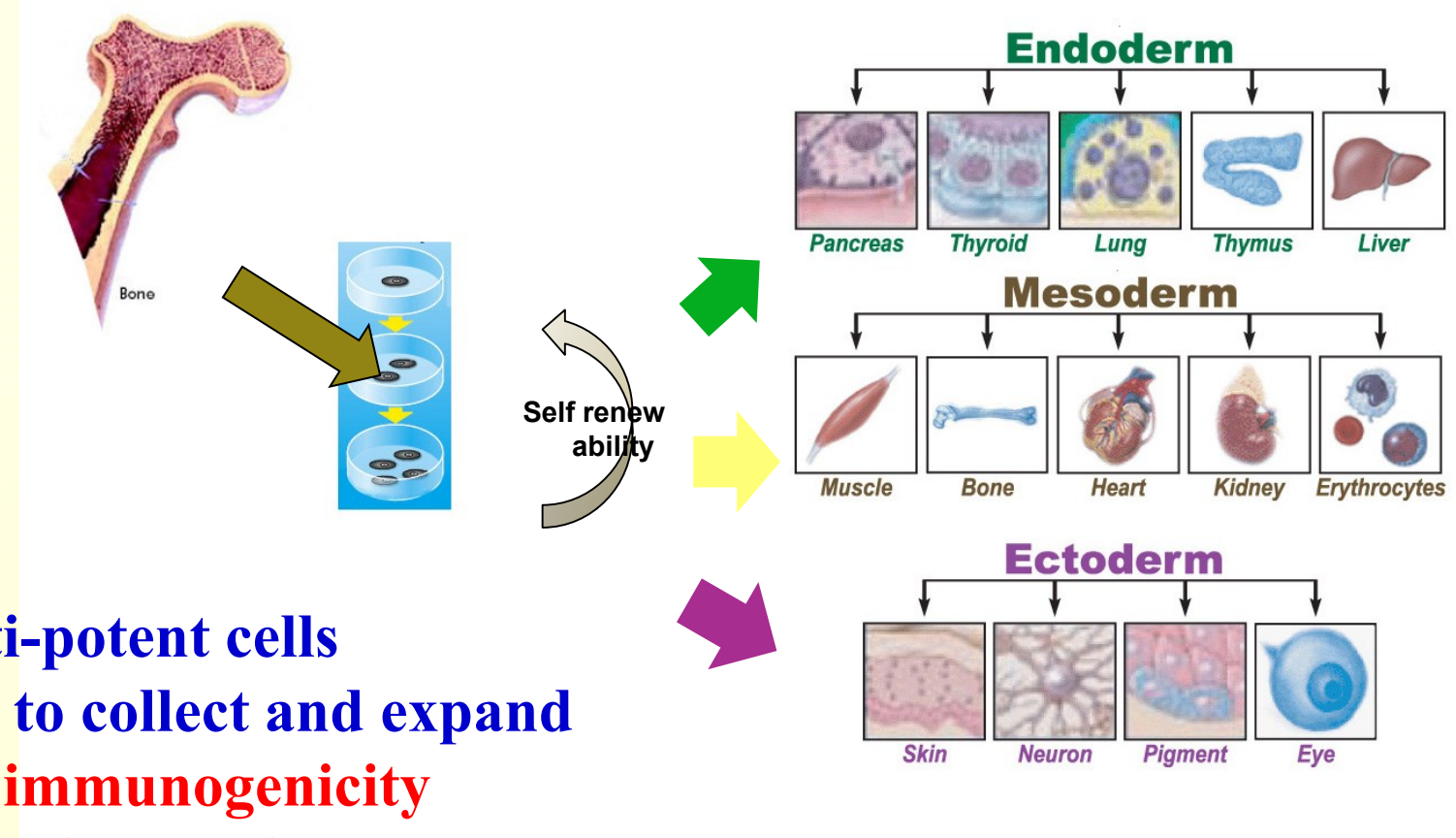
Gang Li

Dept. of Orthopaedics and Traumatology

School of Biomedical Sciences,

The Chinese University Hong Kong

Bone Marrow Mesenchymal Stem Cells (MSCs)



- Multi-potent cells
- Easy to collect and expand
- Low immunogenicity
- Systemic recruitment
- Home to injury tissues

Stem Cells 2007; 25:69-77.

STEM CELLS

TISSUE-SPECIFIC STEM CELLS

Concise Review: Multipotent Mesenchymal Stromal Cells in Blood

QILING HE,[a] CHAO WAN,[b] GANG LI[a]

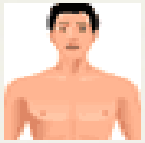
[a]Centre for Cancer Research and Cell Biology, Musculoskeletal Education and Research Unit, School of Biomedical Sciences, Queen's University of Belfast, Musgrave Park Hospital, Belfast, United Kingdom; [b]Department of Pathology, Division of Molecular and Cellular Pathology, University of Alabama at Birmingham, Birmingham, Alabama, USA

Key Words. Peripheral blood • Colony-forming units fibroblastic • Multipotent mesenchymal stromal cells
Peripheral blood-derived multipotent mesenchymal stromal cells

ABSTRACT

Peripheral blood-derived multipotent mesenchymal stromal cells circulate in low number. They share, most although not all, of the surface markers with bone marrow-derived multipotent mesenchymal stromal cells, possess diverse and complicated gene expression characteristics, and are capable of differentiating along and even beyond mesenchymal lineages. Although their origin and physio-pathological function are still unclear, their presence in the adult peripheral blood might relate to some interesting but controversial subjects in the field of adult stem cell biology, such as systemic migration of bone marrow-derived multipotent mesenchymal stromal cells and the existence of common hematopoietic-mesenchymal precursors. In this review, current studies/knowledge about peripheral blood-derived multipotent mesenchymal stromal cells is summarized, and the above-mentioned topics are discussed. STEM CELLS *2007;25:69–77*

➢Progress to date



human

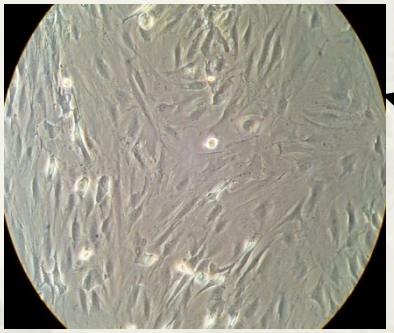
2001. J Cell Biol.

153:1133-1139

Osteogensesis in vivo; Adipogenesis in vitro

CD34-/CD45-/CD14-
CD44+/CD106+ / Type I collagen+
CD105-/Alkaline phosphatase -/ strol-1-

} human cells



PB-derived adherent, clonogenic, fibroblast-like cells

Circulating osteoblast-lineage cells in humans. New Engl. J. Med. May 12, 2005.

- **Sorted osteocalcin+ cells in children**
- **Formed bone in vivo**
- **Increased numbers in three adults with recent fractures.**

CD34 $^{-/low}$
Osteocalcin^{+}

2000. Stem cells

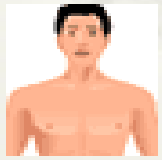
18:252-260

2000. Arthritis Res.

2:477-488

Osteogenesis in vitro

CD34^{-}/CD45^{-}/CD14^{-}/CD3^{-}
CD105^{+}/type I collagen^{+}

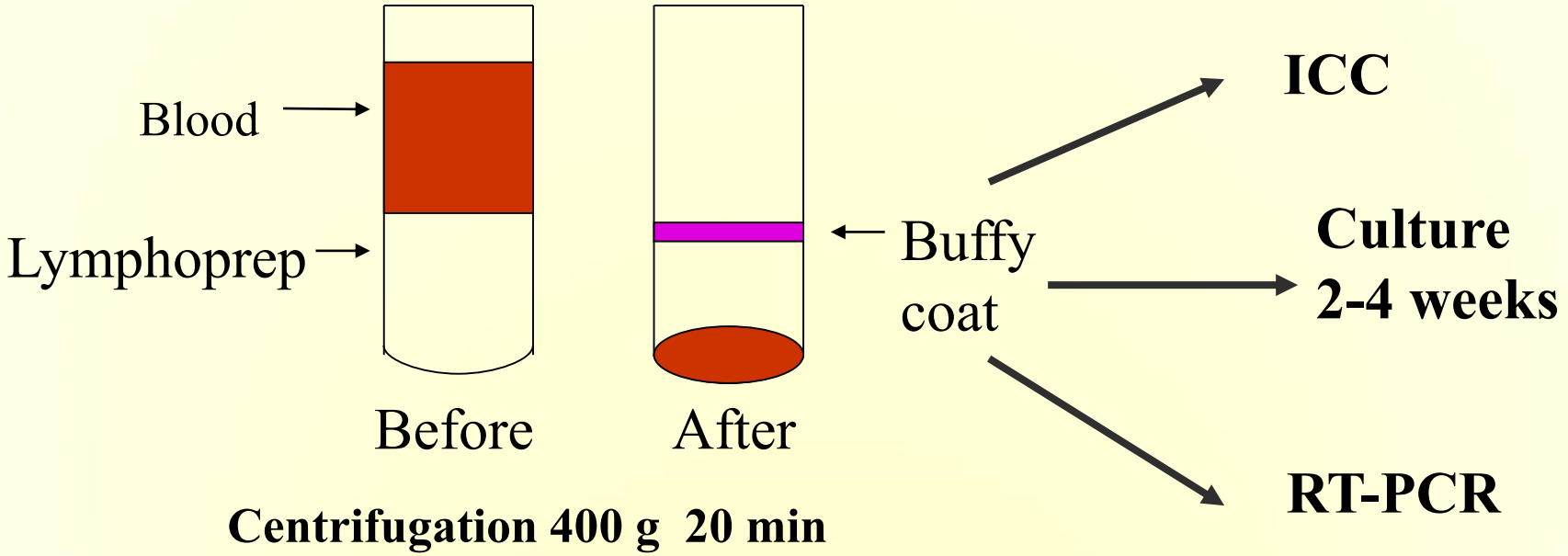


human

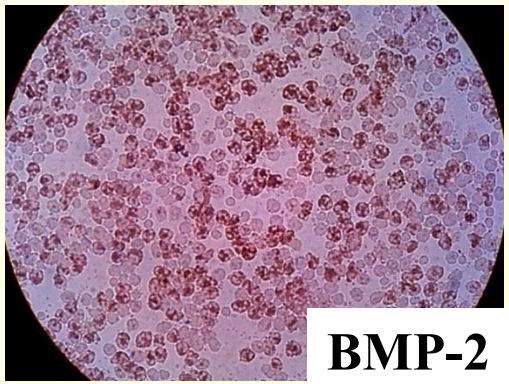
Study of Circulating MSCs in Fracture Patients

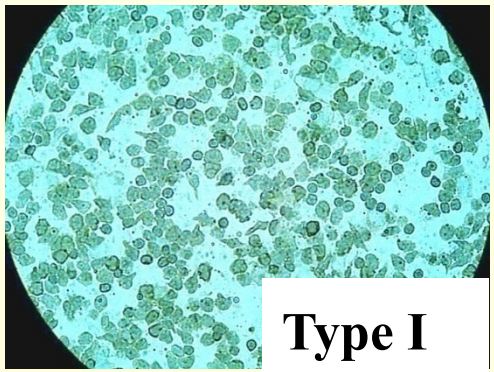
30 mls of peripheral blood collected from 8 fracture patients, at 3 time-points after fracture (days 1-3, 9-12 and 16-21) and also from 3 normal volunteers and 3 established non-union

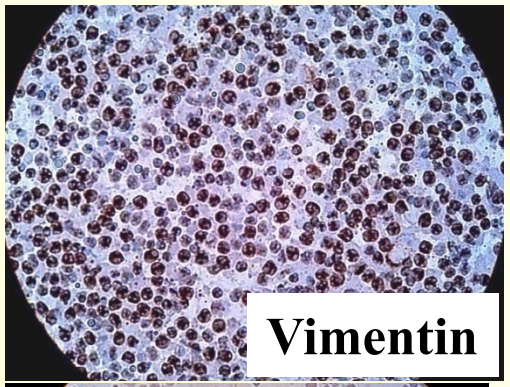
Peripheral blood mononuclear cells (PBMNCs) isolated using LymphoPrep™ density-gradient-centrifugation procedure.

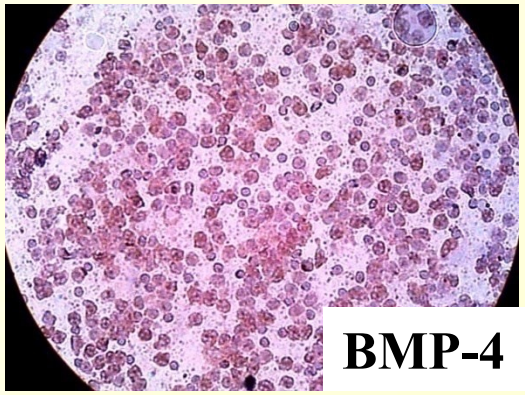


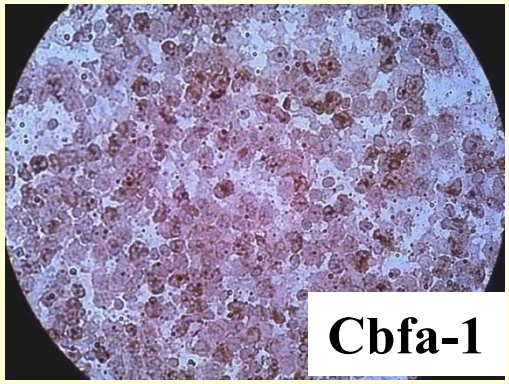
Immunostaining profile of the PBMNCs from a patient with tibial shaft fracture, at day 3 post- fracture

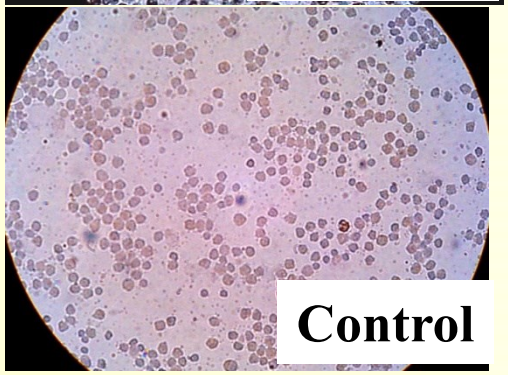


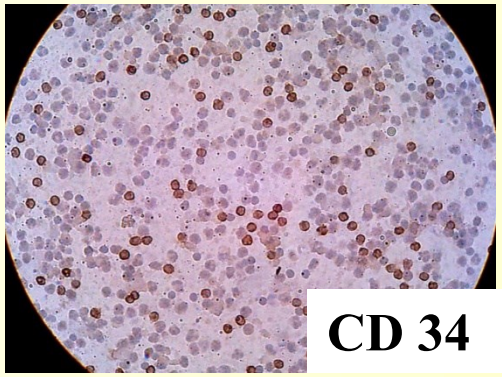


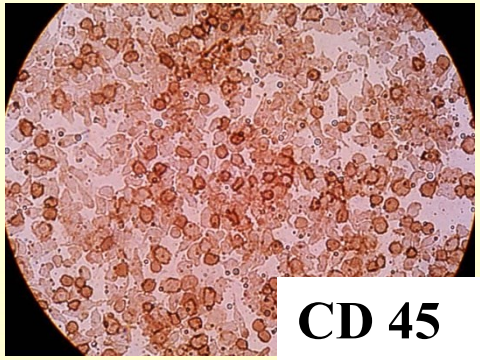


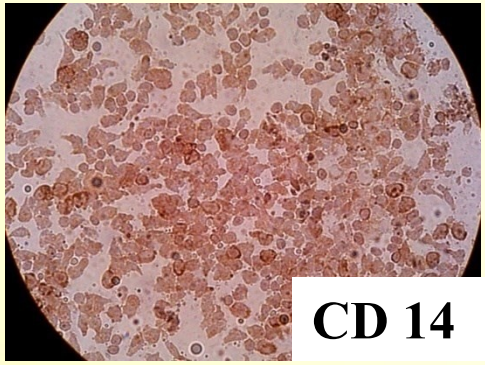




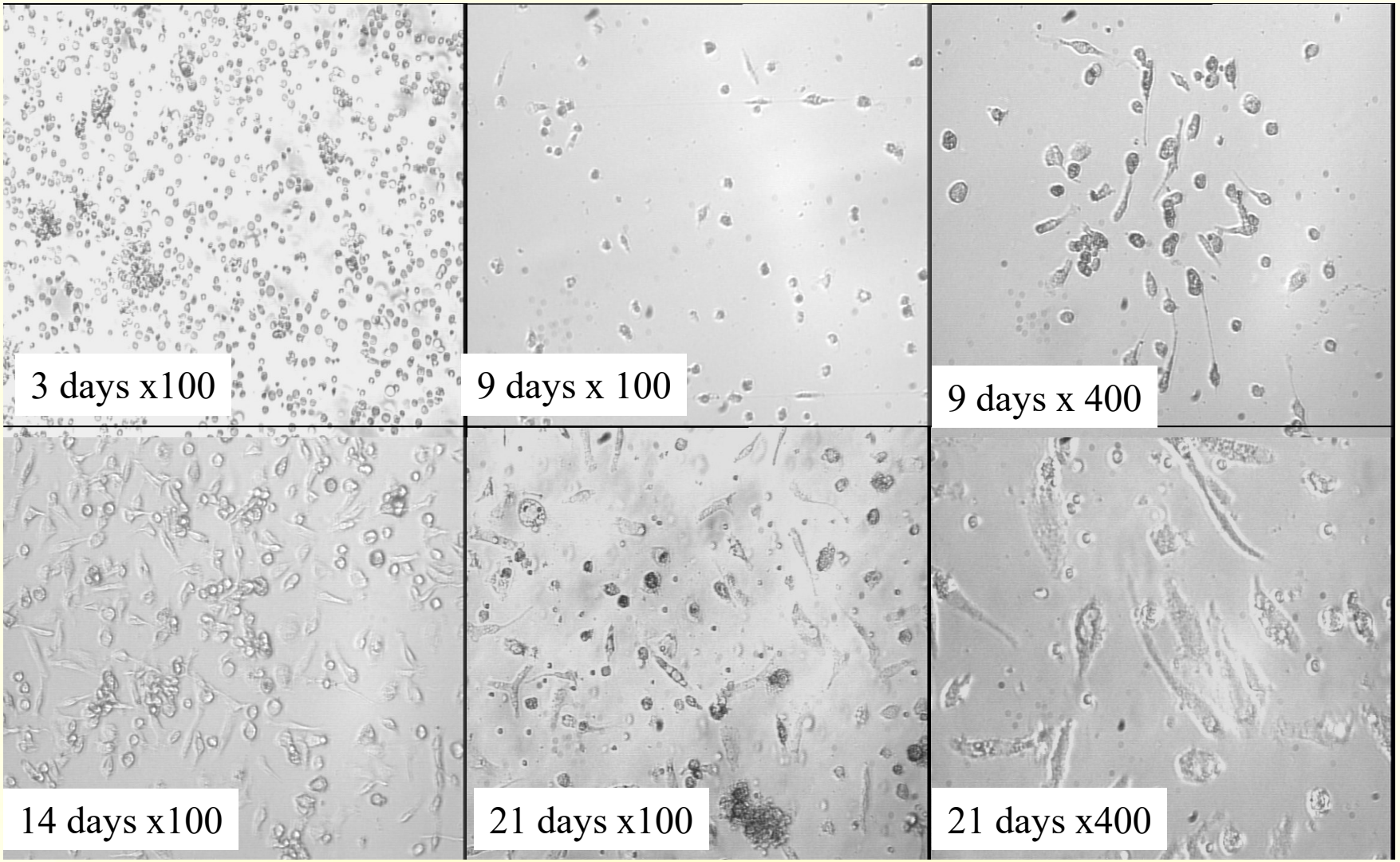




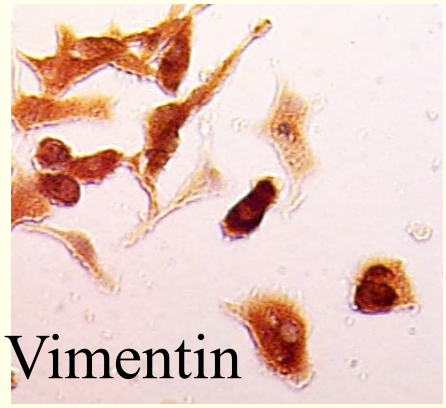


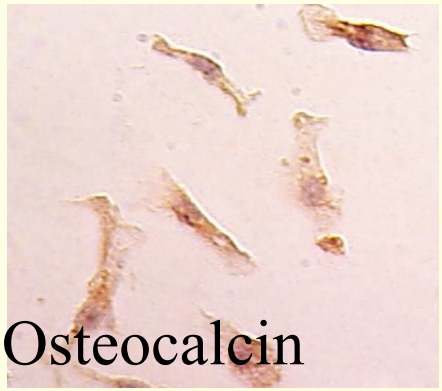


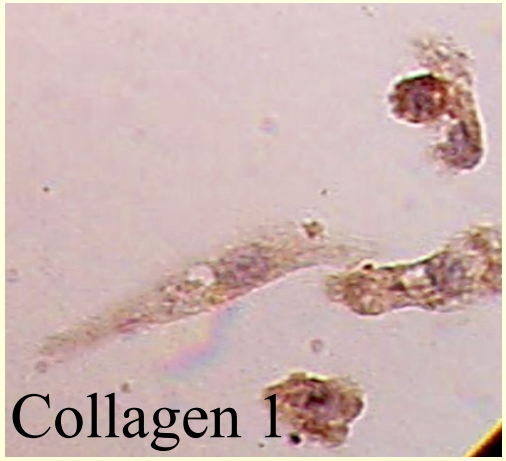
PBMNCs in Culture with osteogenic medium from a patients with tibial fracture, day 16

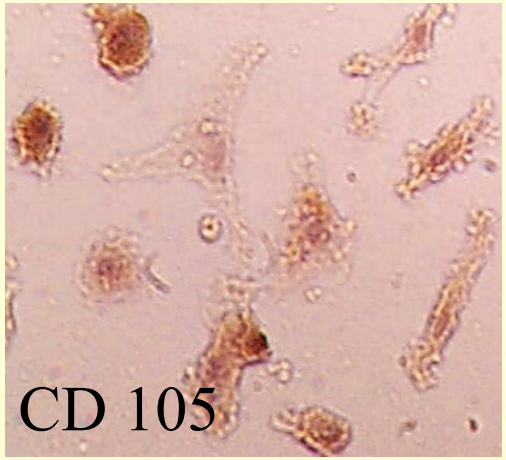


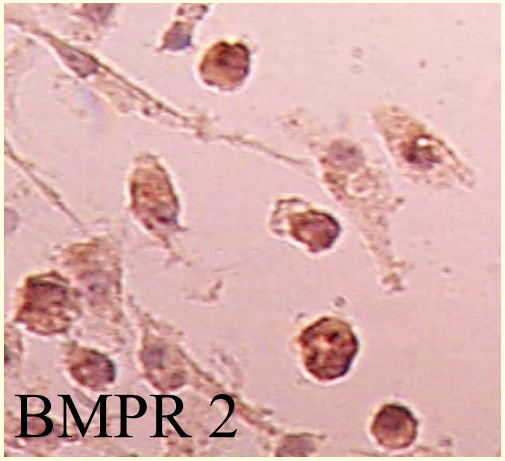
Immunocytochemistry on a human fracture patient's PBMNC culture at 2 weeks

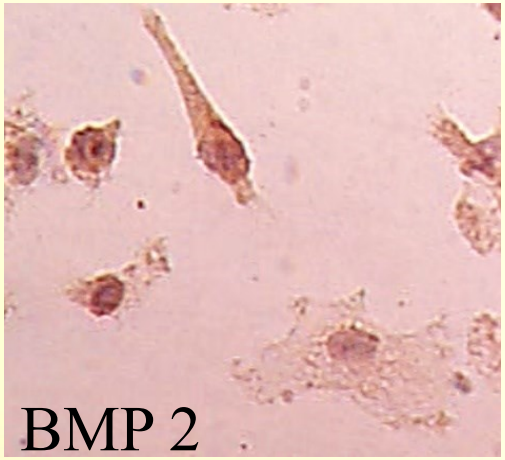




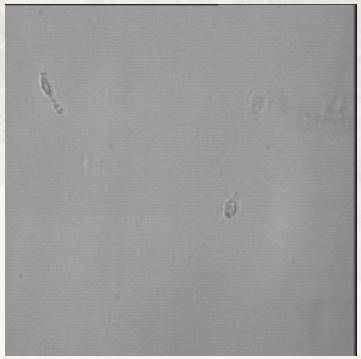
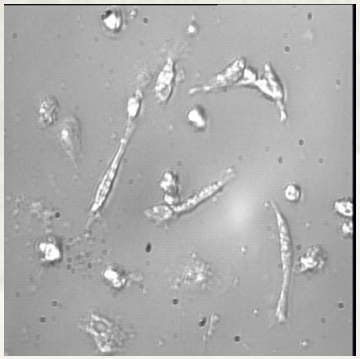
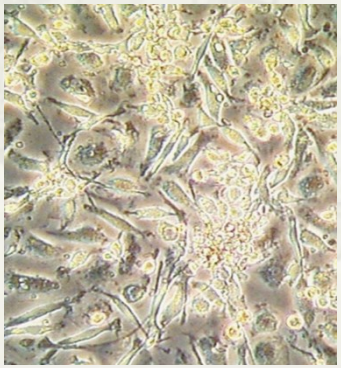
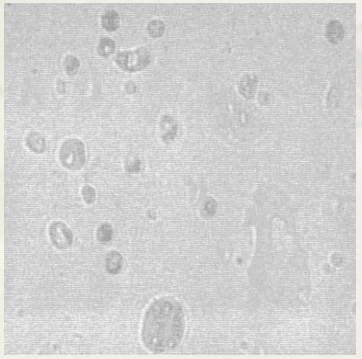








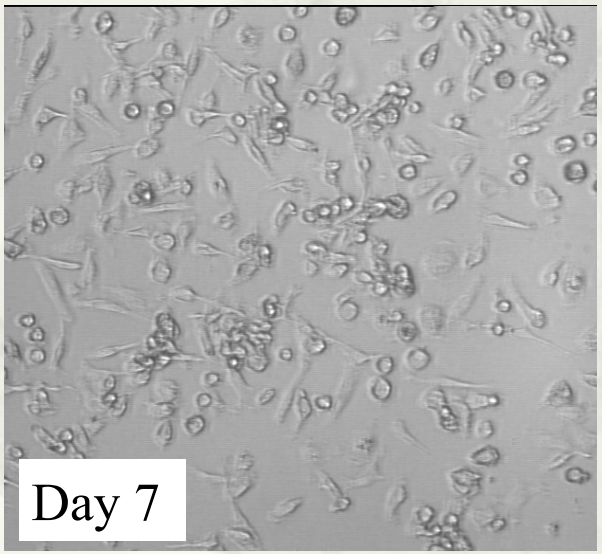
Summary of cell culture results

	< 4 days Post-fracture	> 14 days Post-fracture	Non-union patients	Control
cases	5	5	3	4
cells	few 	Some 	Many 	None/few 

➢ *In search of blood borne MSCs*

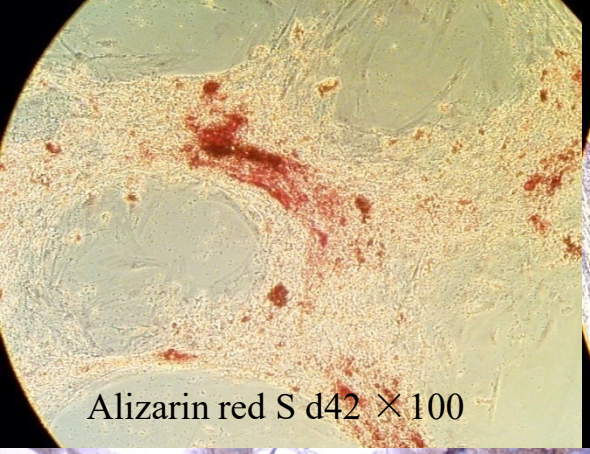
Normal Adult Peripheral Blood

- 1 MSC in ~ 10^9 MNCs in normal adult peripheral blood
- (*vs.* 1 MSC in 10^6 bone marrow) MNCs
- Numbers of MSCs increased in patients with fracture

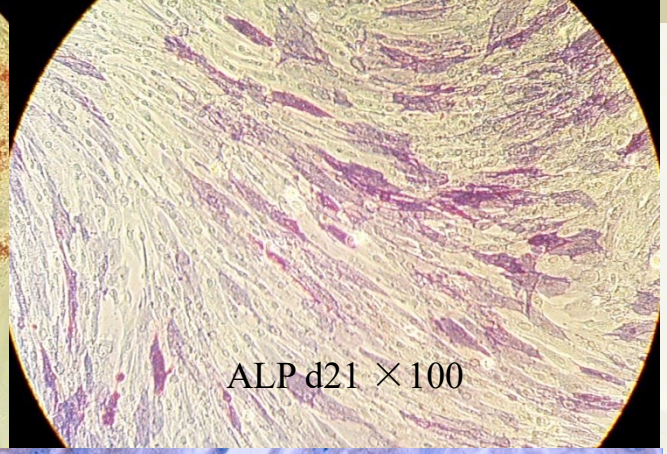


Greater numbers of spindle/polygonal cells fund in the peripheral blood MNCs from the patients with fracture non-union, suggesting a systemic recruitment of MSCs may exist (*Shirley, et al. J. Orthop. Res. 2005: 23 (5): 1013-21*)

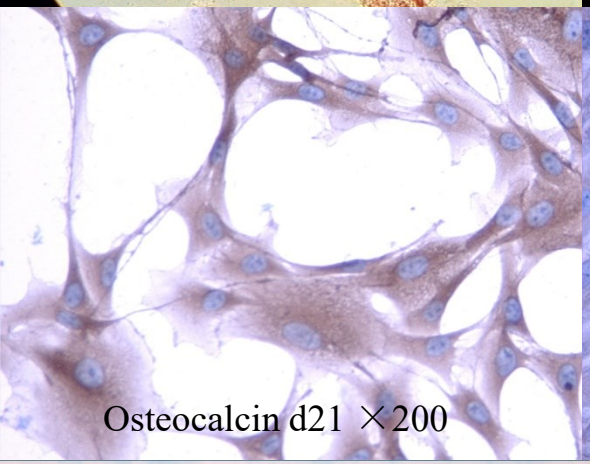
Characterization of MSCs from non-union patients blood



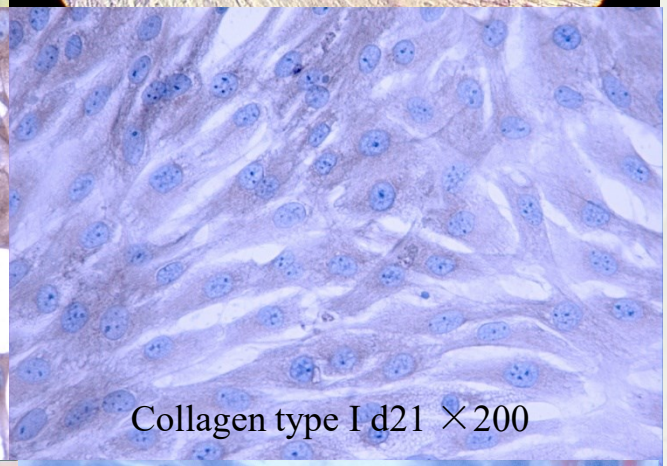
Alizarin red S d42 ×100



ALP d21 ×100

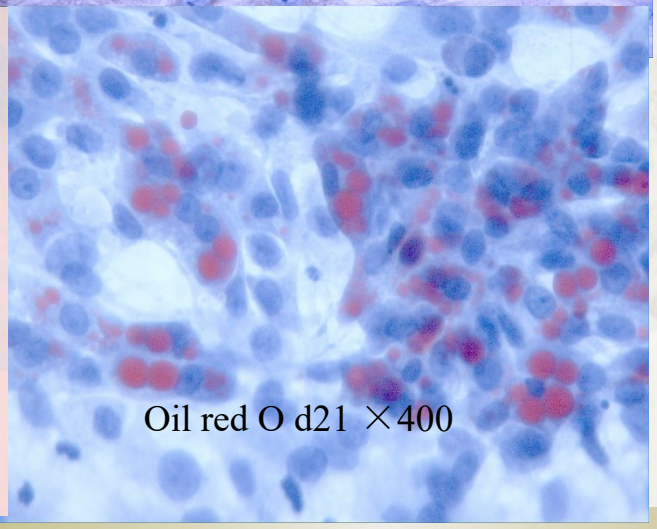


Osteocalcin d21 ×200

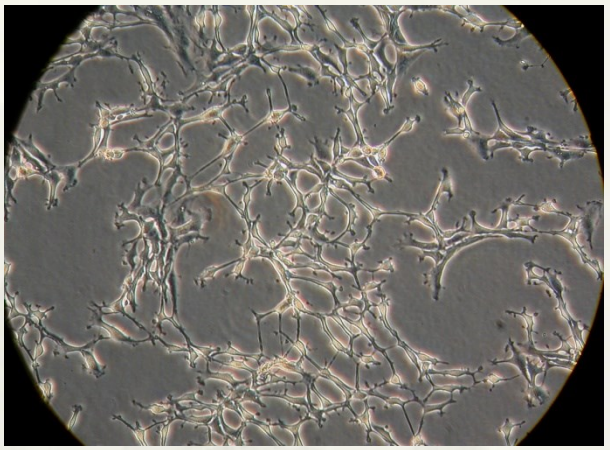


Collagen type I d21 ×200

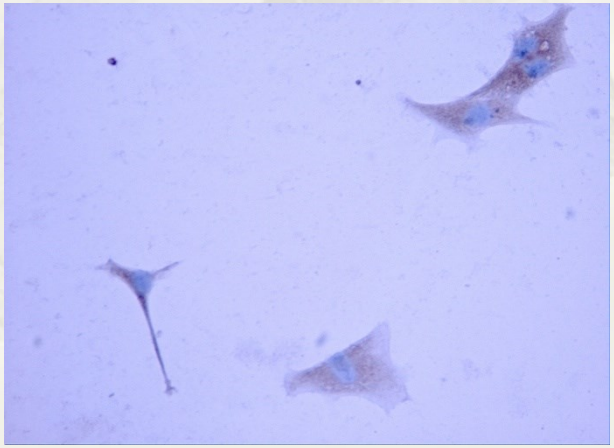
Alcian blue d21



Oil red O d21 ×400

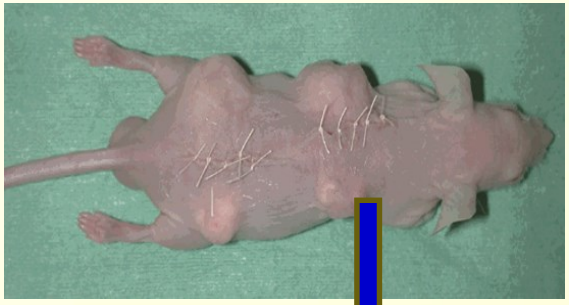


β-ME 6h ×100

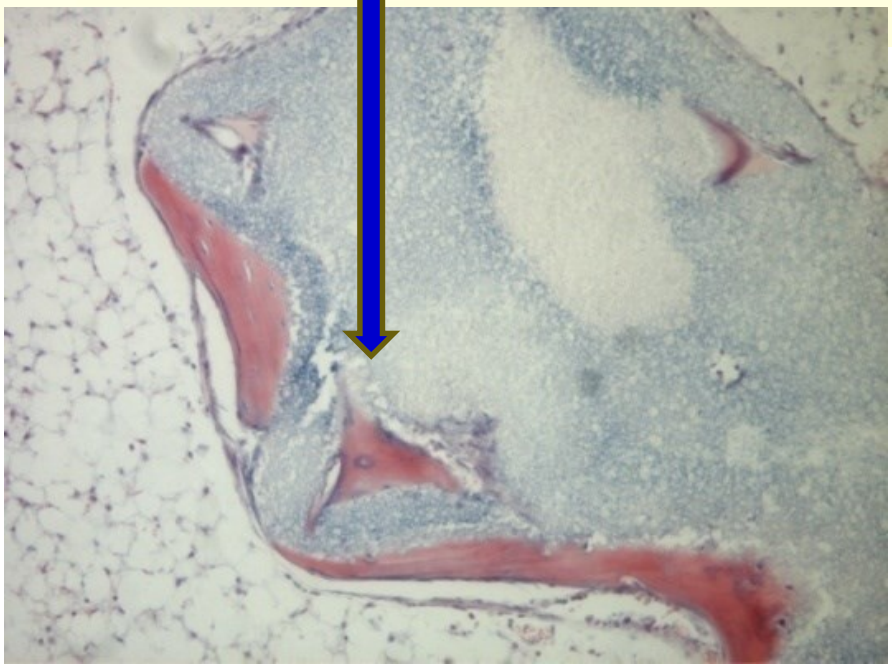


Neurofilament β-ME 6h ×200

Differentiation Potential of human blood-borne MSCs



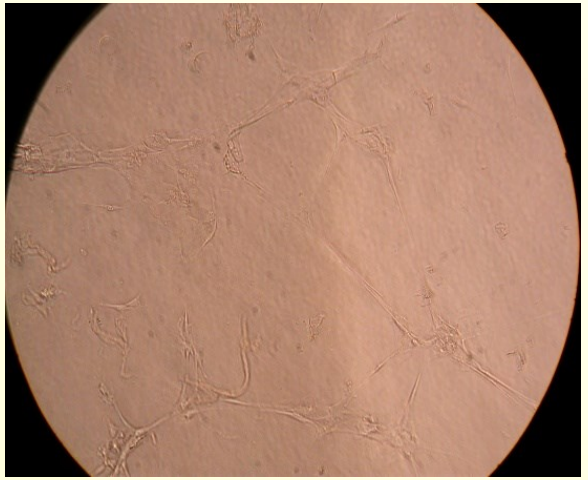
In vivo bone formation study



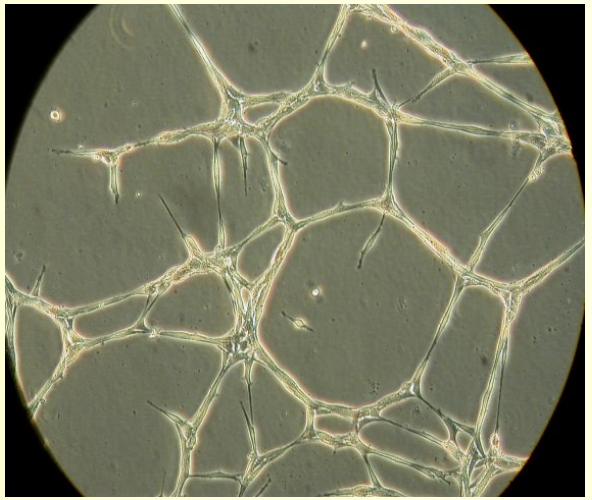
-

PBMSCsseeded CaP block 3month×50

In vitro angiogenesis



Matrigel 3D culture 24h ×100



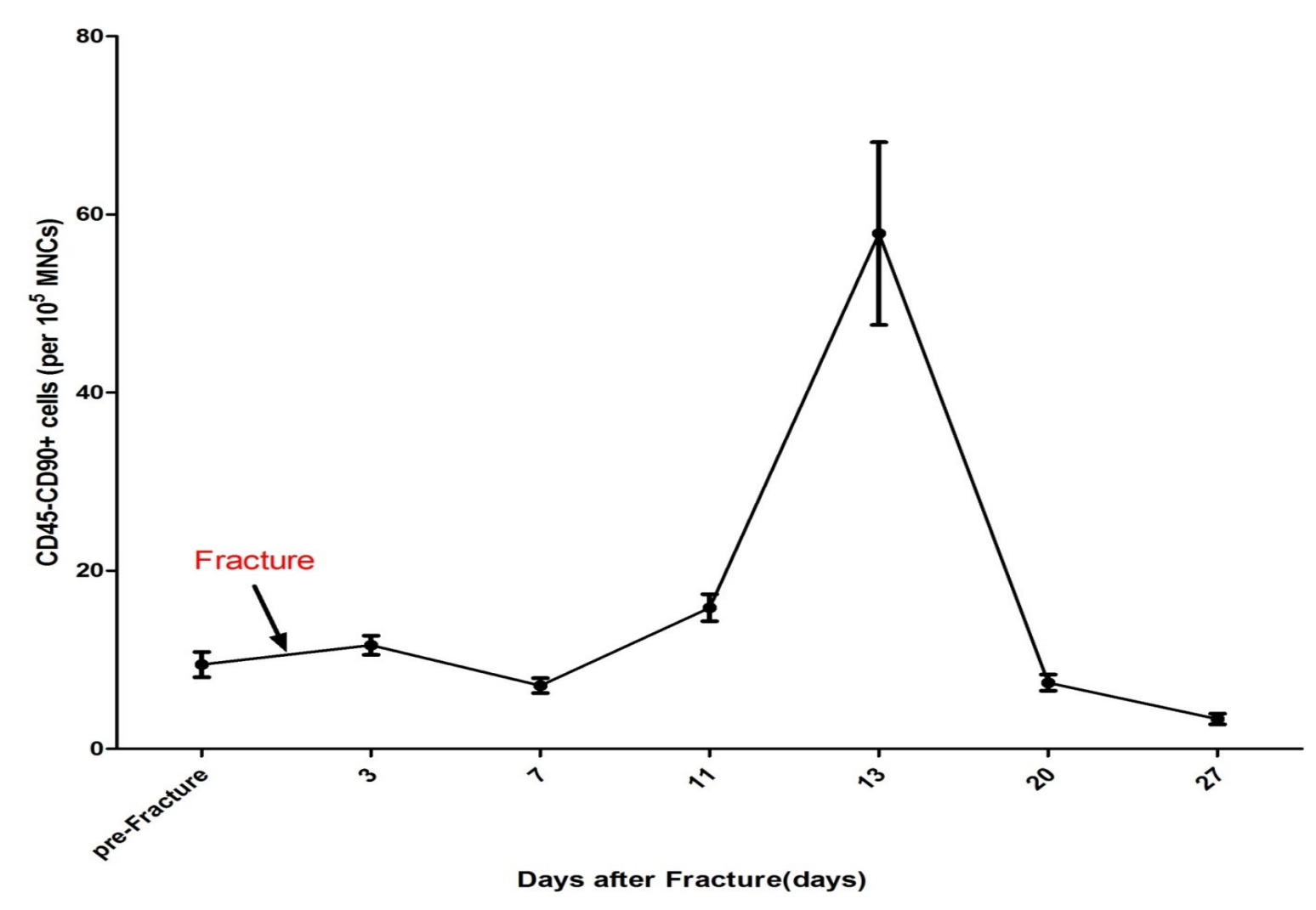
Long term 2D culture 72h ×100

Change of circulating MSCs during fracture healing in rats

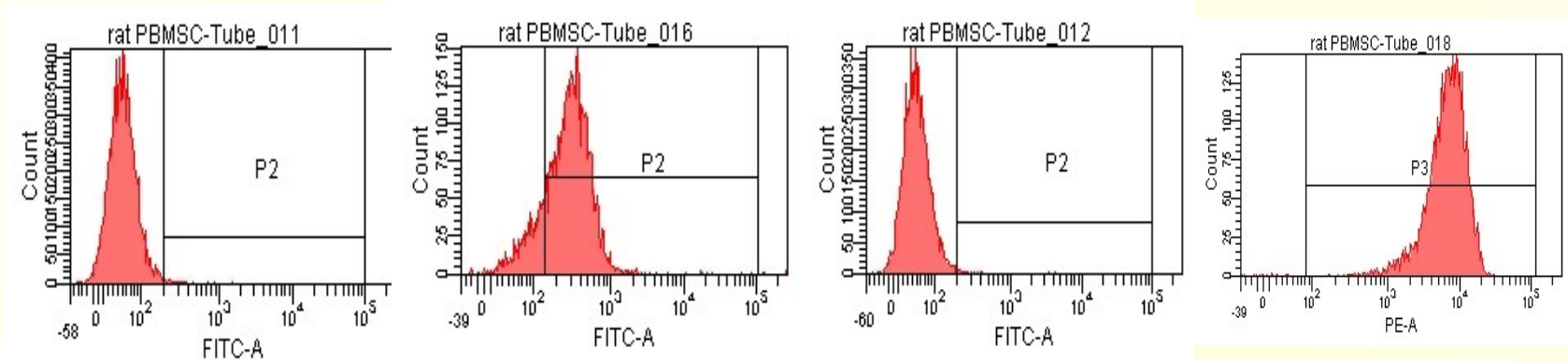
* Femoral closed fracture was created in 12 male SD rats (age 12 weeks) with intramedullary nail fixation.

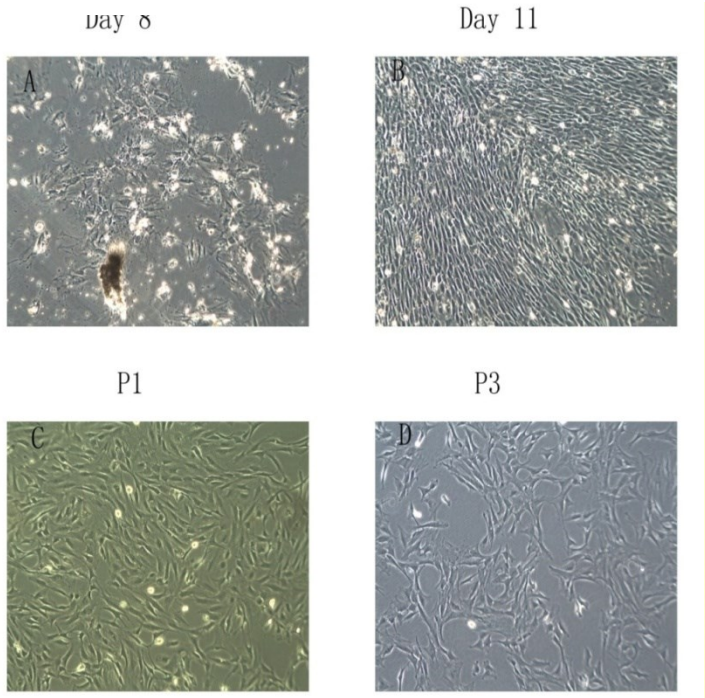
* 0.5 ml Peripheral blood was taken from the eye vein at day before fracture, 3, 7, 11, 13, 20, 27 post fracture; CD45 and CD 90 were used to labeled the cells as representative markers for circulating MSCs and subject to flowcytometry analysis.

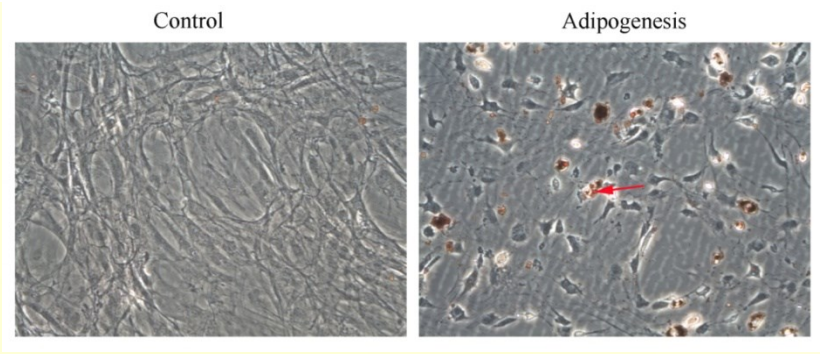
Results: Changes of blood MSCs (CD45-CD90+) during fracture process

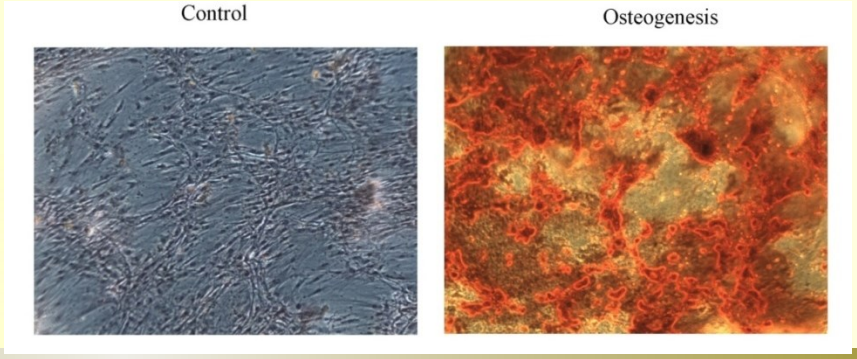


Results: Characterization and differentiation potentials of Circulating MSCs

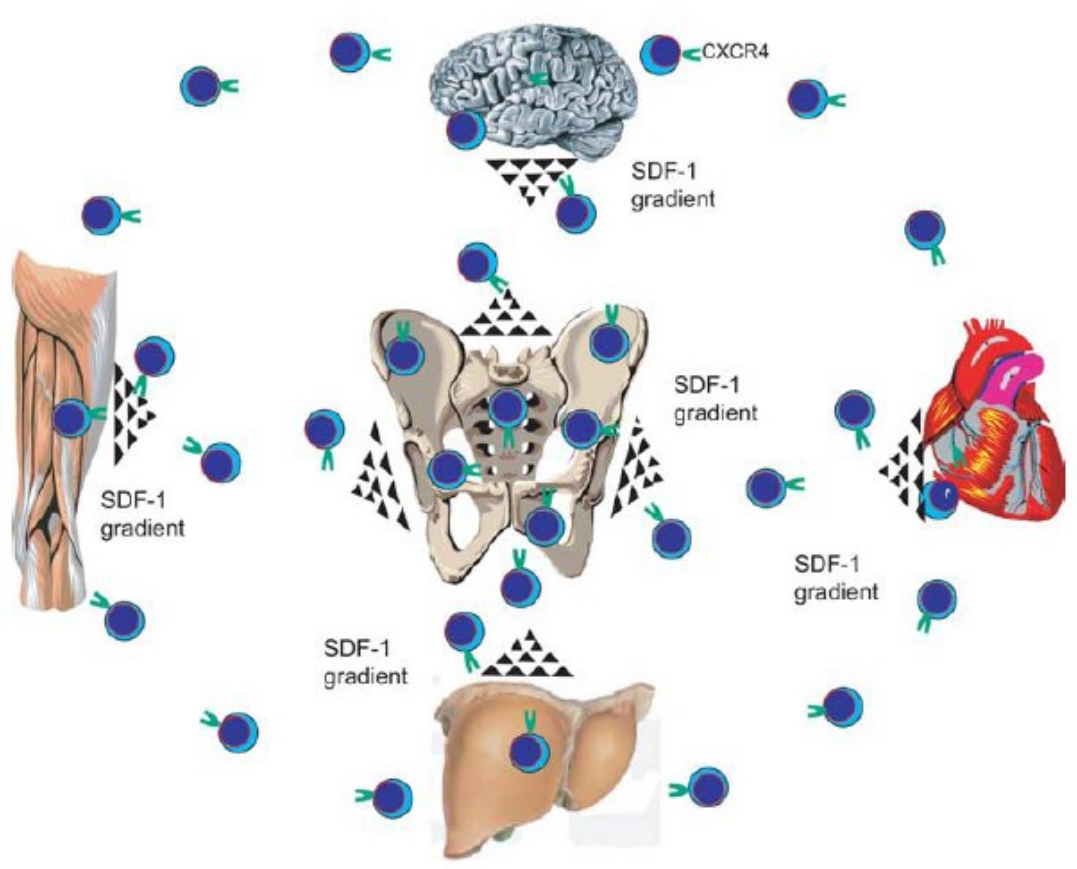








MSCs Home to Injury Sites



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.

Where do circulating MSCs come from?

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,*]

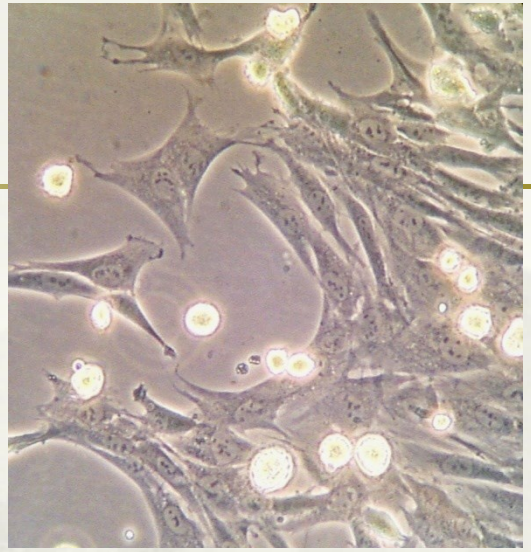
[a] *Department of Trauma and Orthopaedic Surgery, School of Medicine, Queen's University Belfast, Musgrave Park Hospital, Belfast BT9 7JB, UK*

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

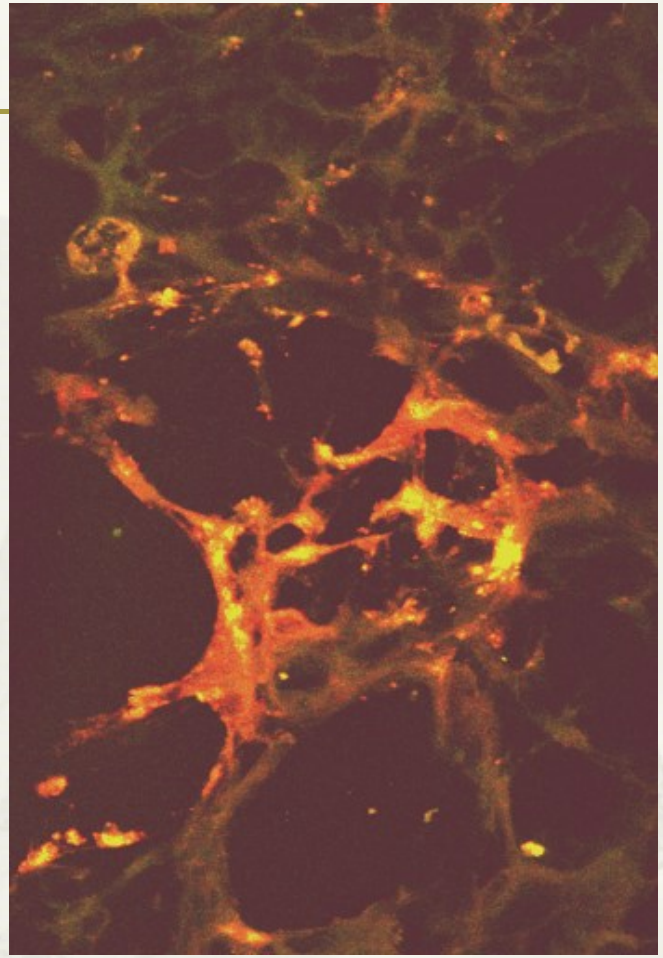
Accepted 28 January 2005

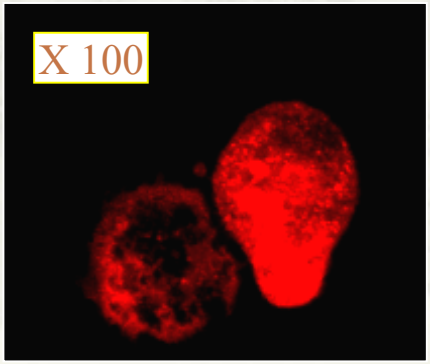
MSCs homes to fracture sites through peripheral circulation

Bone marrow harvested



Rabbit bone marrow
MSCs culture





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

Re-implantation

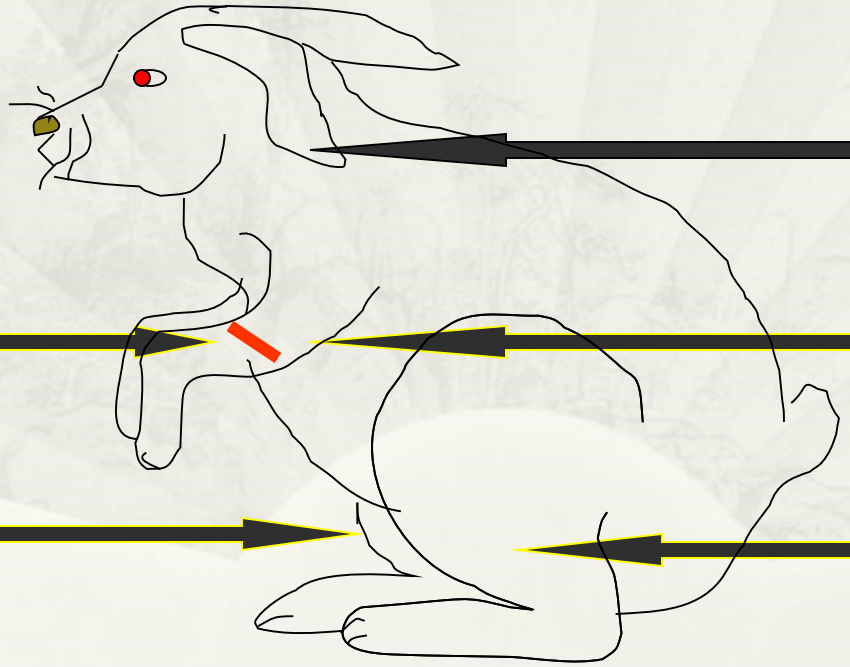
48 hours post Fx

In each group some animals were sacrificed at 3 & 12 weeks

Culture 3 weeks

Ulnar defect

BM harvest



Control E

Ear vein B

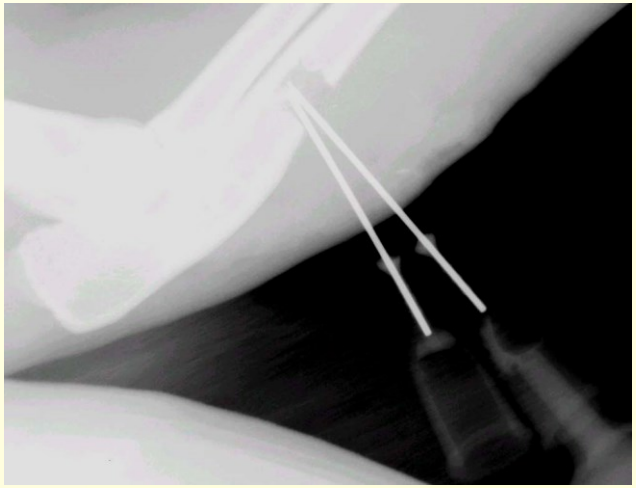
Fracture site A

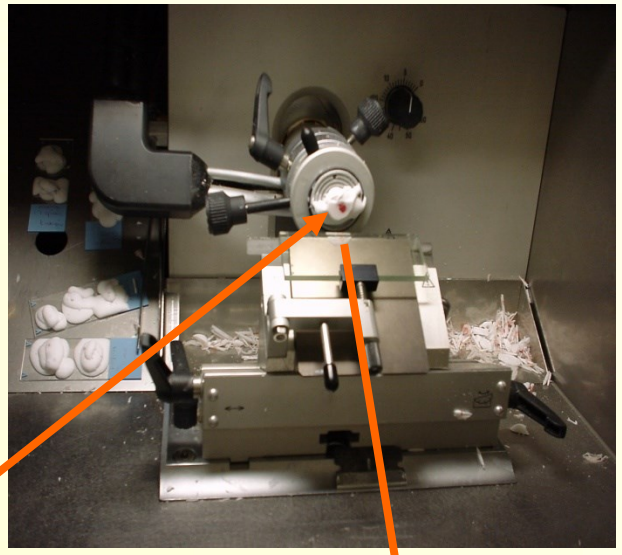
Remote BM site C

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

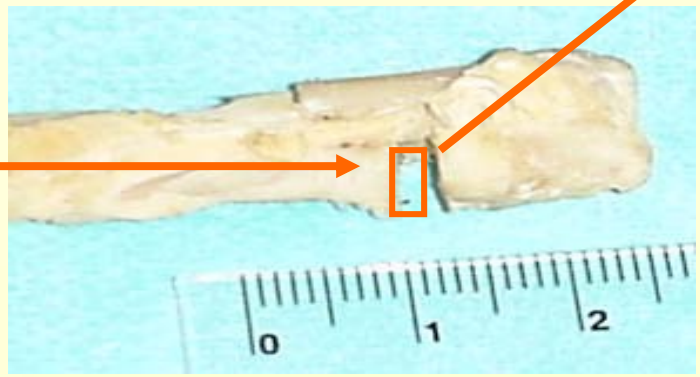
The tissues retrieved for frozen section – (5μm)

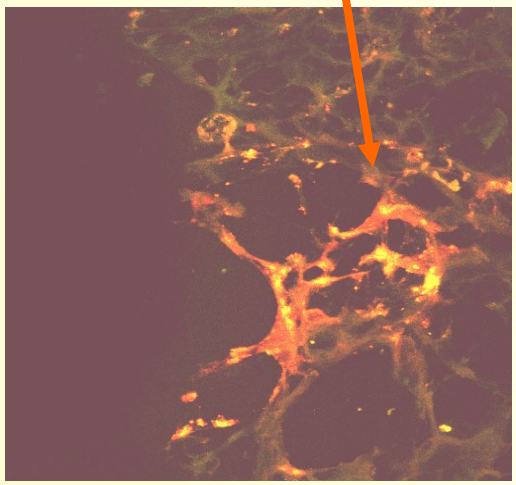
Animals were sacrificed at 3 and 12 weeks after cell implantation





Gap tissue



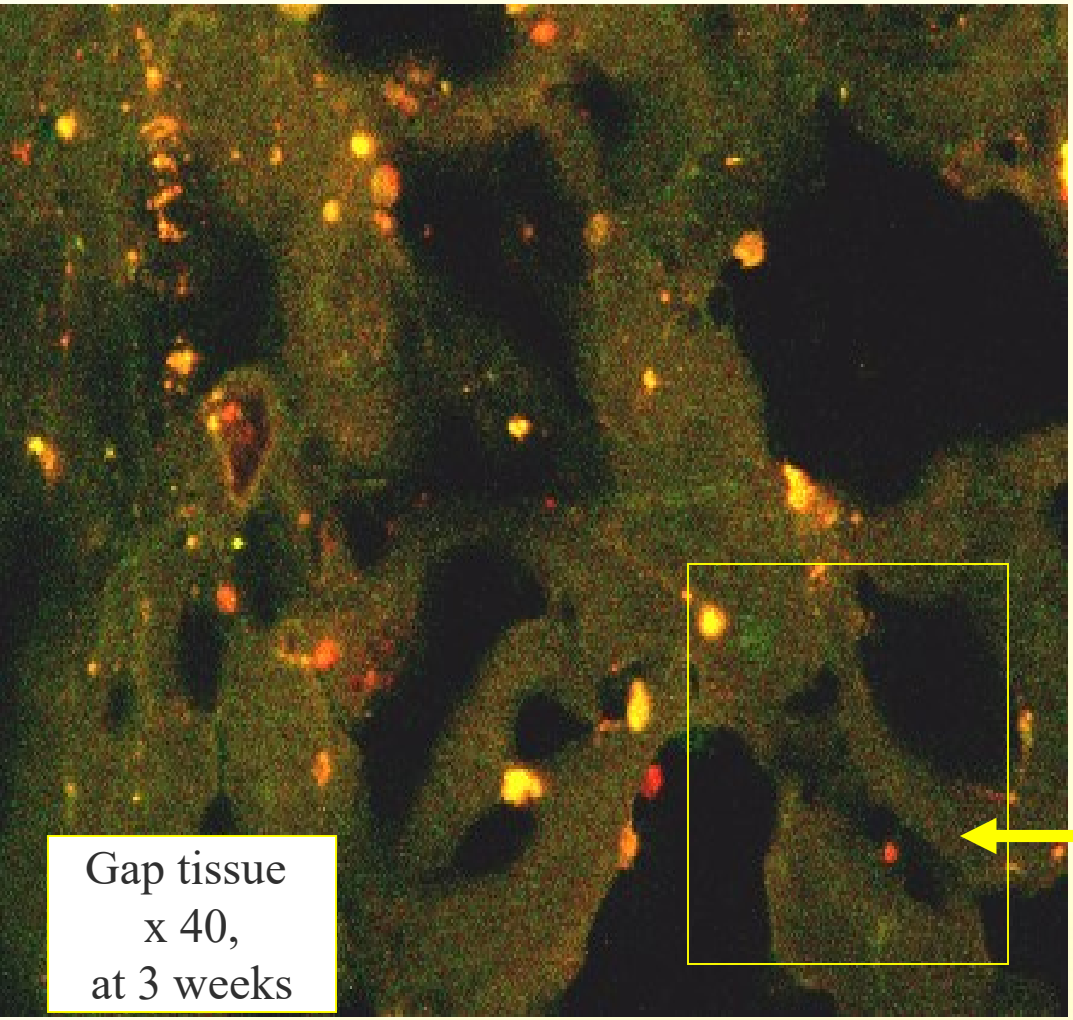


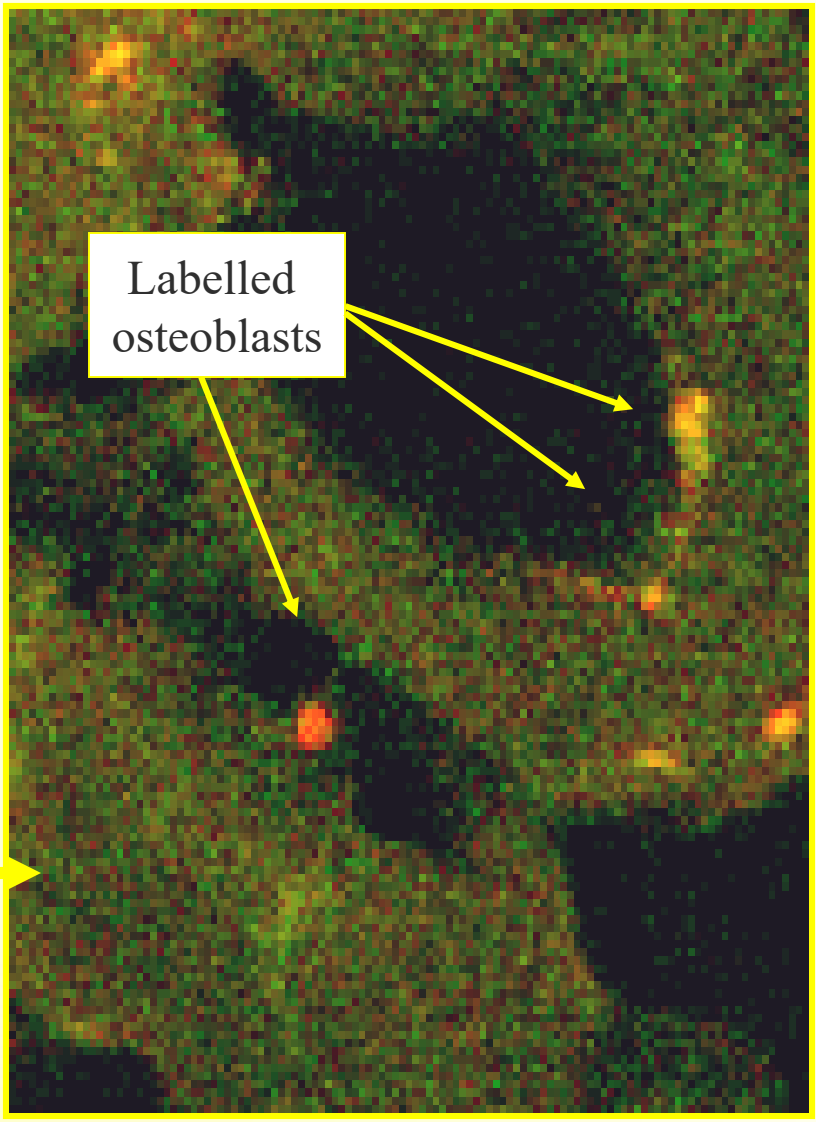
– Liver, lung, kidney, and spleen,

– Also cytospins of BM and blood

(representative samples only)

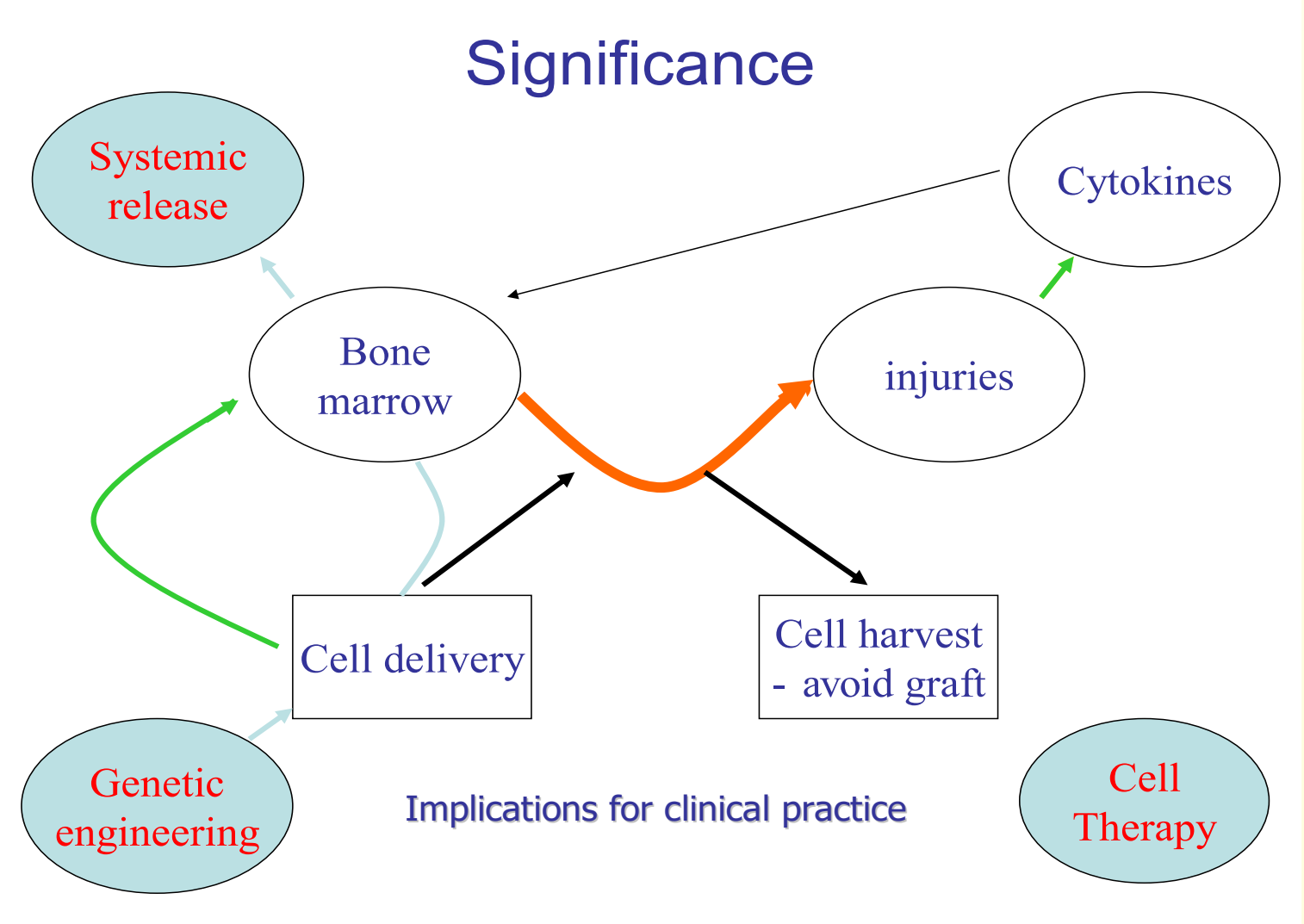
Labelled cells from remote marrow identified at the fracture gap (Group with systemic injection of allogenic MSCs)





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

- **Some osteoblasts integral in fracture repair come from remote bone marrow sites.**
- **They were actively recruited through the peripheral circulation.**



Local *Vs.* Systemic MSCs Administration

- **Local injection of autologous MSCs have been shown to promote fracture healing (Chanda et al, 2010; Li, et al 2010) .**

- **3-4 weeks time is needed to culture-expend MSCs to sufficient therapeutic numbers, may miss the "window of opportunities".**

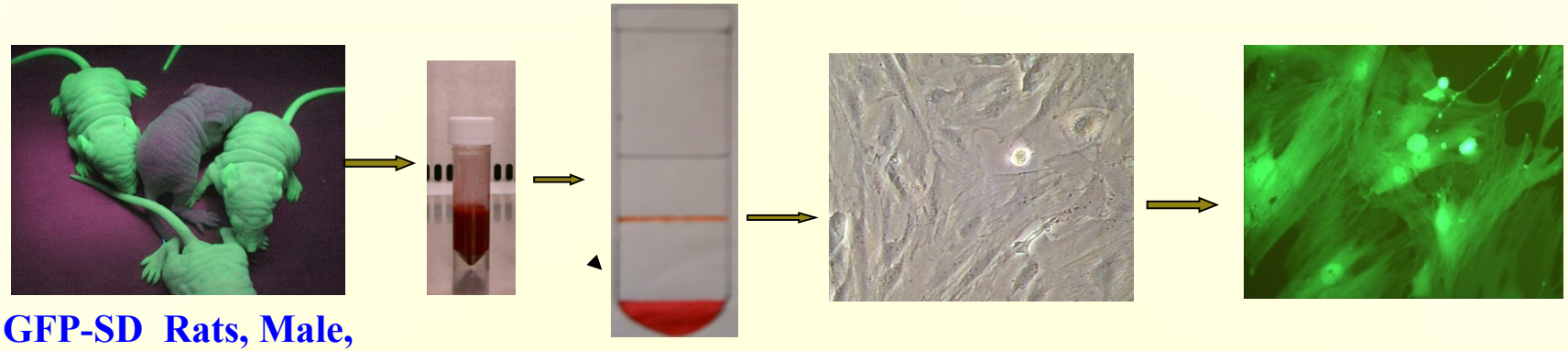
Local *Vs.* Systemic MSCs Administration

- **Locally delivered MSCs often face hostile microenvironment: lack of blood supply; infection; inflammation that minimize their survival and impair their function *in vivo*.**

- **Systematic administrated MSCs may reach the fracture sites through circulation, where the sufficient blood supply will enhance their survival and function.**

Materials and Methods

Cell Preparation

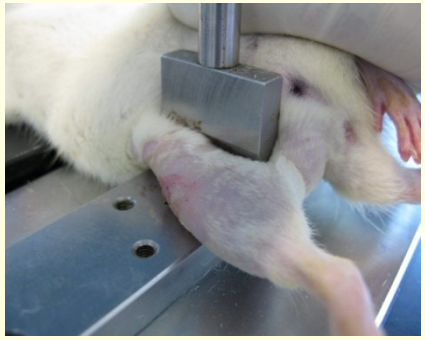


GFP-SD Rats, Male,
3-6 month old

- Isolation of BM-MSCs and skin fibroblasts from GFP-Rat
- Flow cytometry analysis for cell surface antigen markers:
  Positive: CD44, CD73, CD90, CD146
  Negative: CD31, CD34, CD45
- Differentiation assays: adipogenesis, osteogenesis, chondrogenesis

Materials and Methods

Animal Experimental Groups

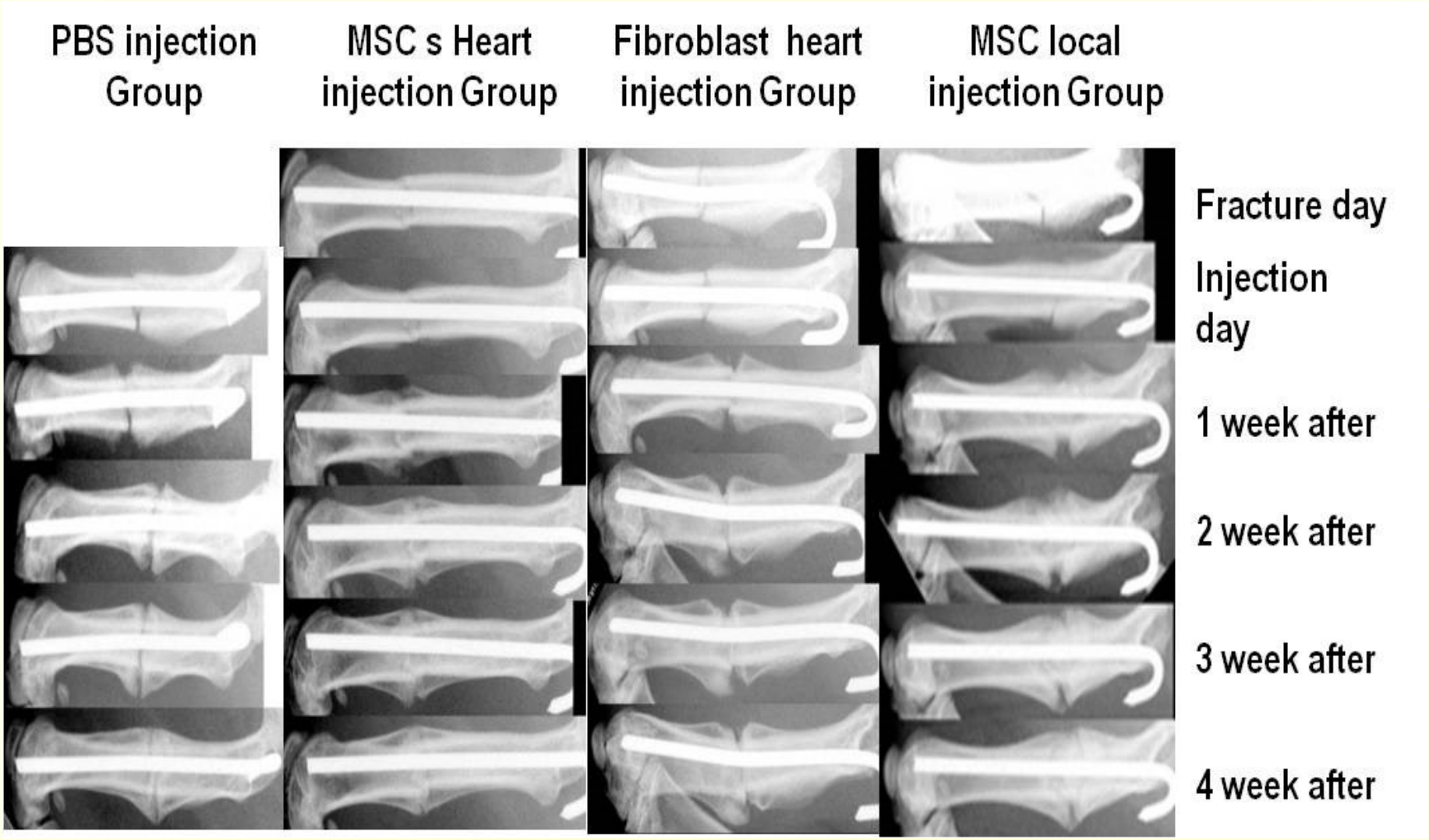

- 48 male SD rats (age: 12 weeks) had right femoral closed fracture
- Fracture was fixed with intramedullary nail
- Animals were randomly assigned into 4 experimental groups (n=12)

PBS Heart Injection Group (Control)	0.5ml PBS/ Rat was given at 4 days post-fx
MSCs Heart Injection Group	$2x10^6$ GFP-MSCs in 0.5ml PBS/ Rat was given at 4 days post-fracture
Fibroblast Heart Injection Group	$2x10^6$ GFP-Fibroblasts in 0.5ml PBS/ Rat was given at 4 days post-fracture
MSCs Fracture Site Injection Group	$2x10^6$ GFP-MSCs in 0.5ml PBS/ Rat was given at 4 days post-fracture

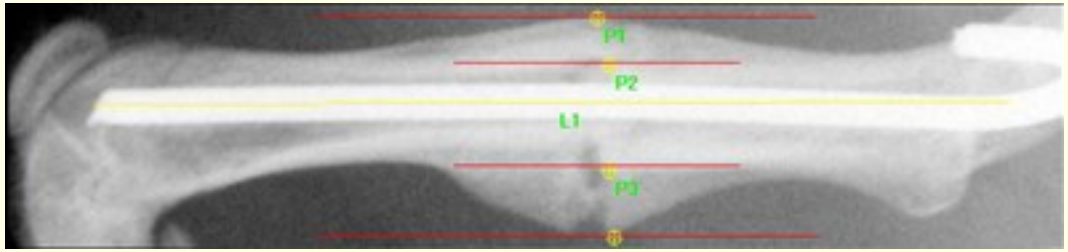
Outcome Measurements

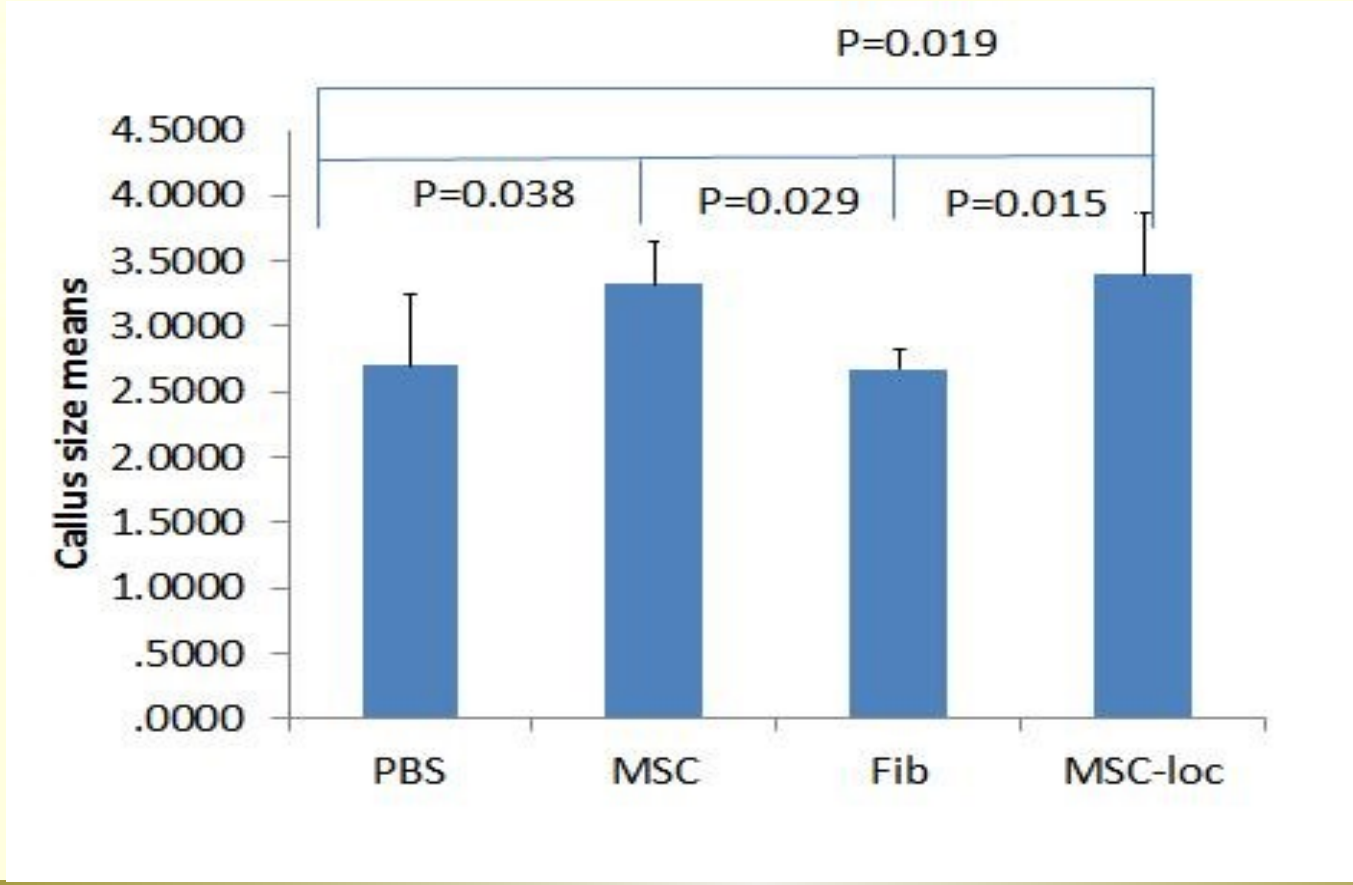
- Weekly body weight and X-ray .
- Terminated at 5 weeks post fracture, both femurs were harvested.
- Micro-CT examination followed by four-point bending mechanical testing.
- Histology and immunohistochemistry examinations.

RESULTS: X-Ray



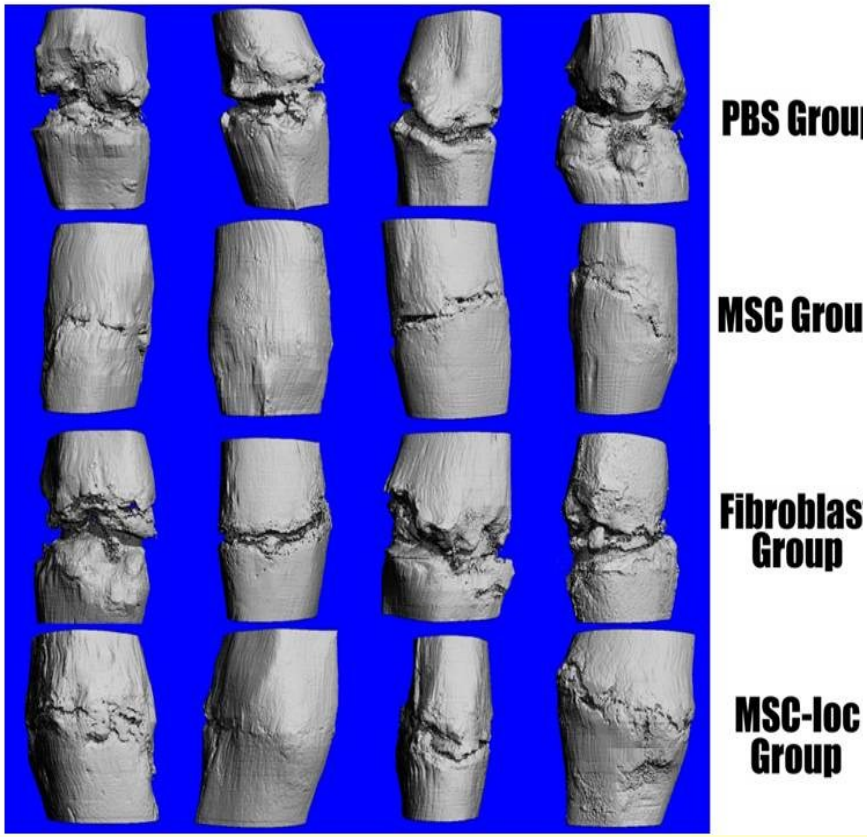
RESULTS- COMPARING THE SIZE OF THE CALLUS



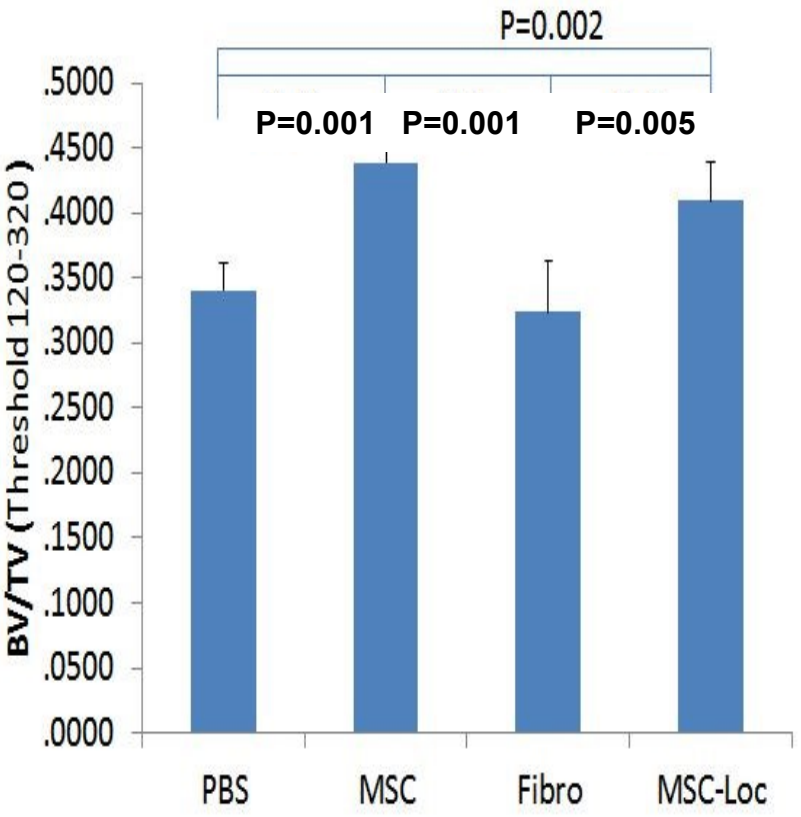


RESULTS: MICRO CT ANALYSIS

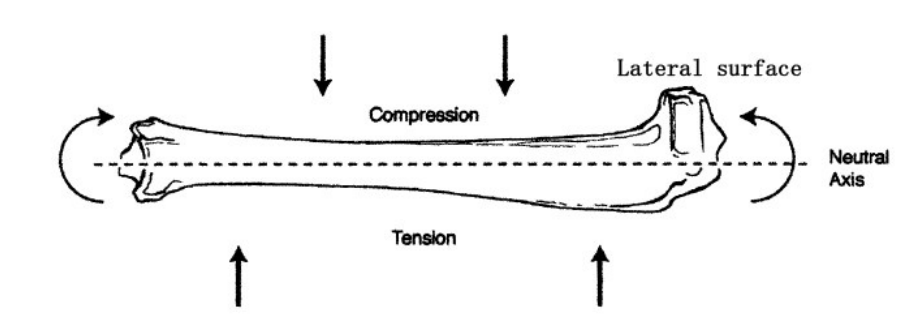
3D Reconstruction

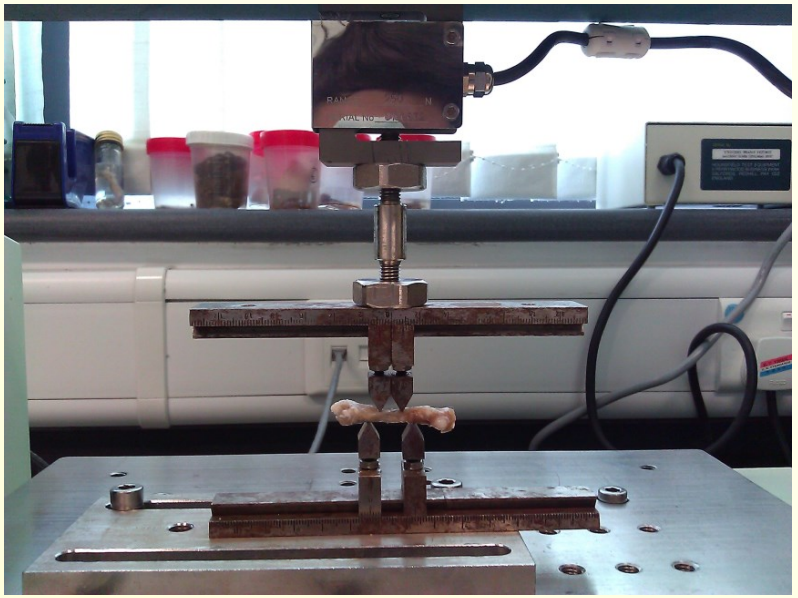


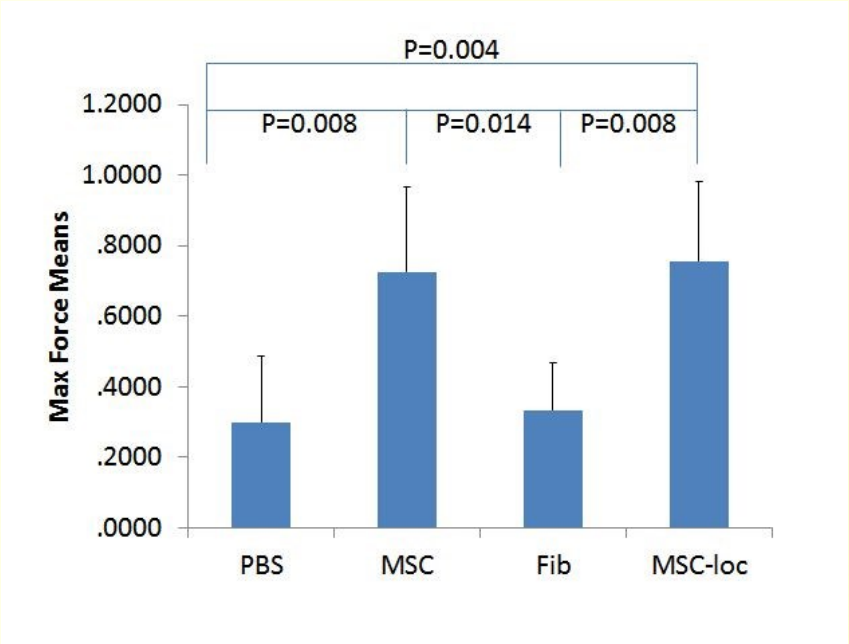
Bone Volume / Total Volume (BV/TV)

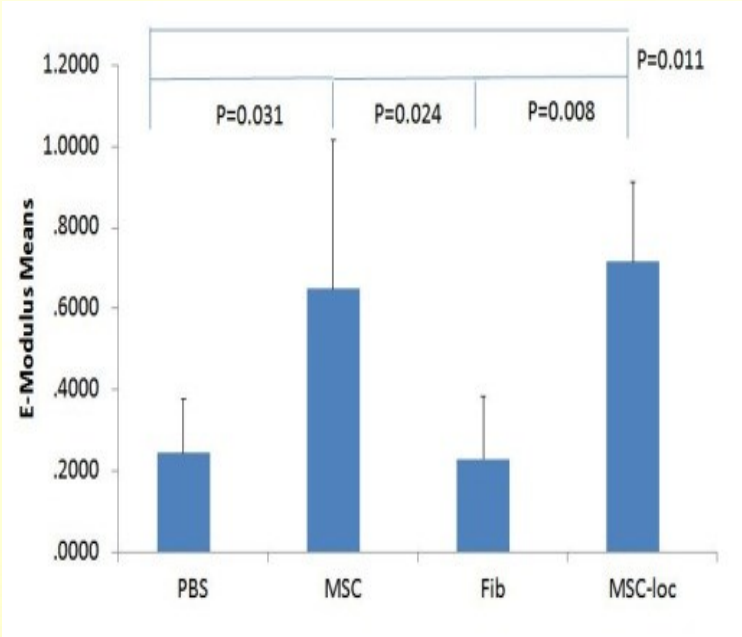


Results: Four-point Bending Mechanical Testing







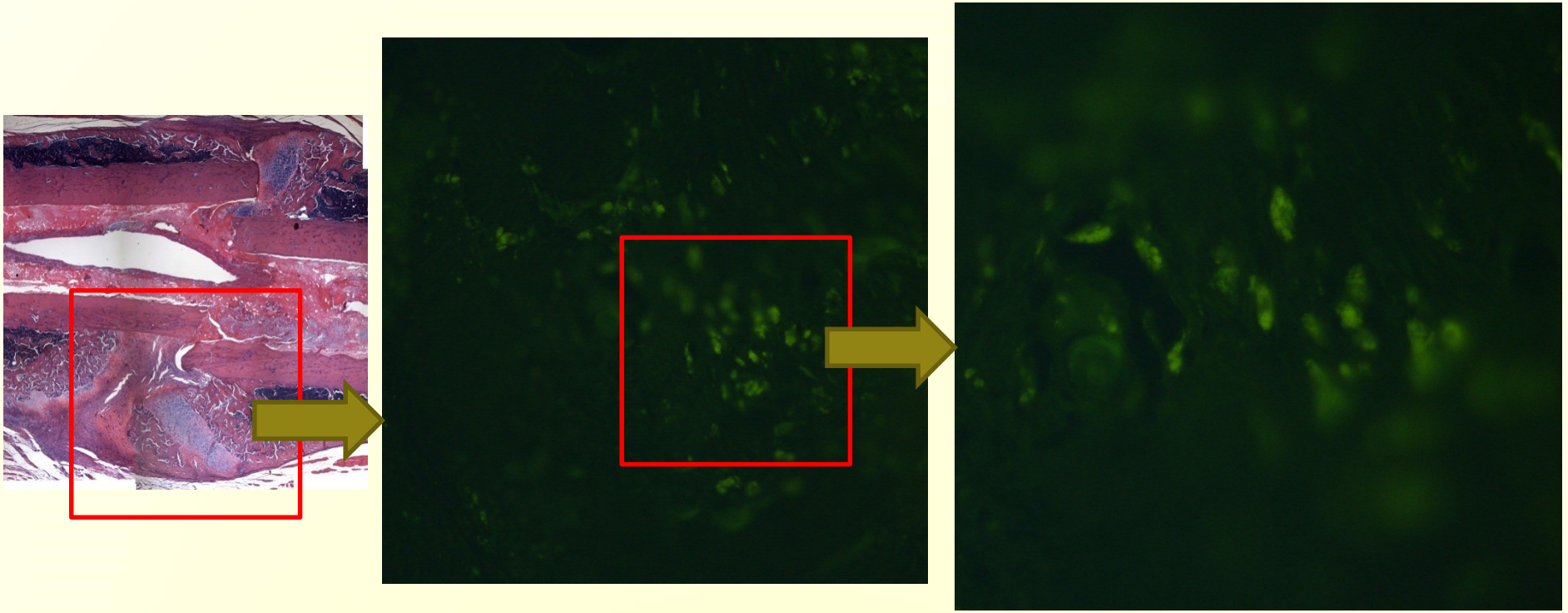


Max Force

E-Modulus (Stiffness)

Results: Histology & Immunofluorescence for GFP-MSCs

MSC systemic injection group



GFP-positive cells were found at the fracture gap 4 weeks following the systemic GFP-MSCs injection.

SUMMARY

- **Both systemic and local injection of allogeneic MSCs promoted bone fracture healing, through enhancing the callus size and biomechanical properties.**

- **The MSCs were present at the fracture site and participated in fracture healing at 4 weeks following their systemic injection. The underlying mechanisms need further investigations.**

CONCLUSION

- **Our findings provide insight for developing systemic administration of allogenic MSCs as a novel therapy strategy for patients with poor fracture healing conditions, such as multiple or high-energy fractures.**

Department of Orthopaedics and Traumatology
CUHK

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