Techniques for Adult Mesenchymal Stem Cell Research and Application

干细胞技术

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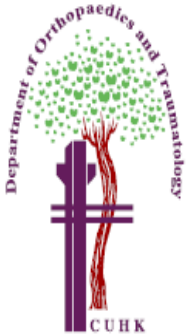

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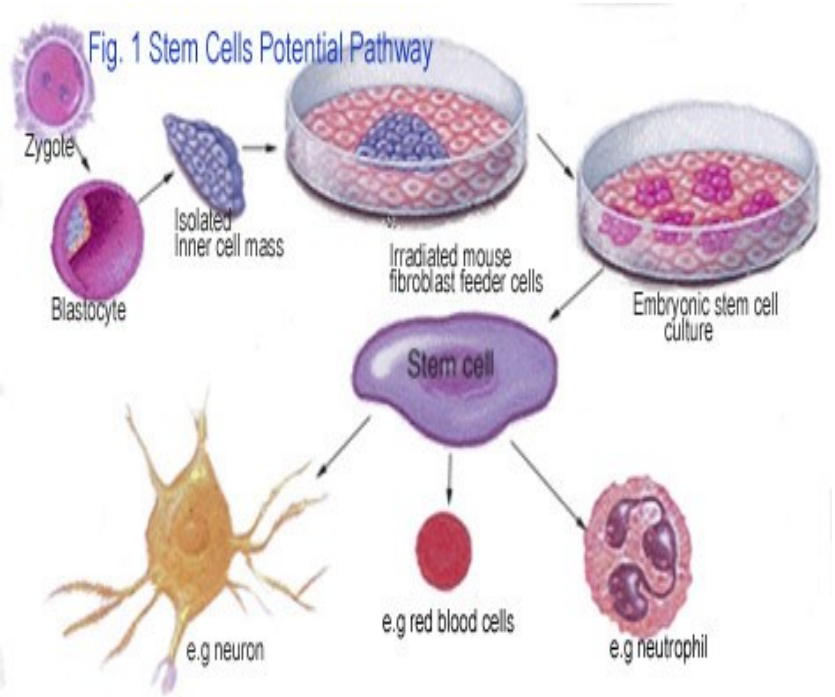
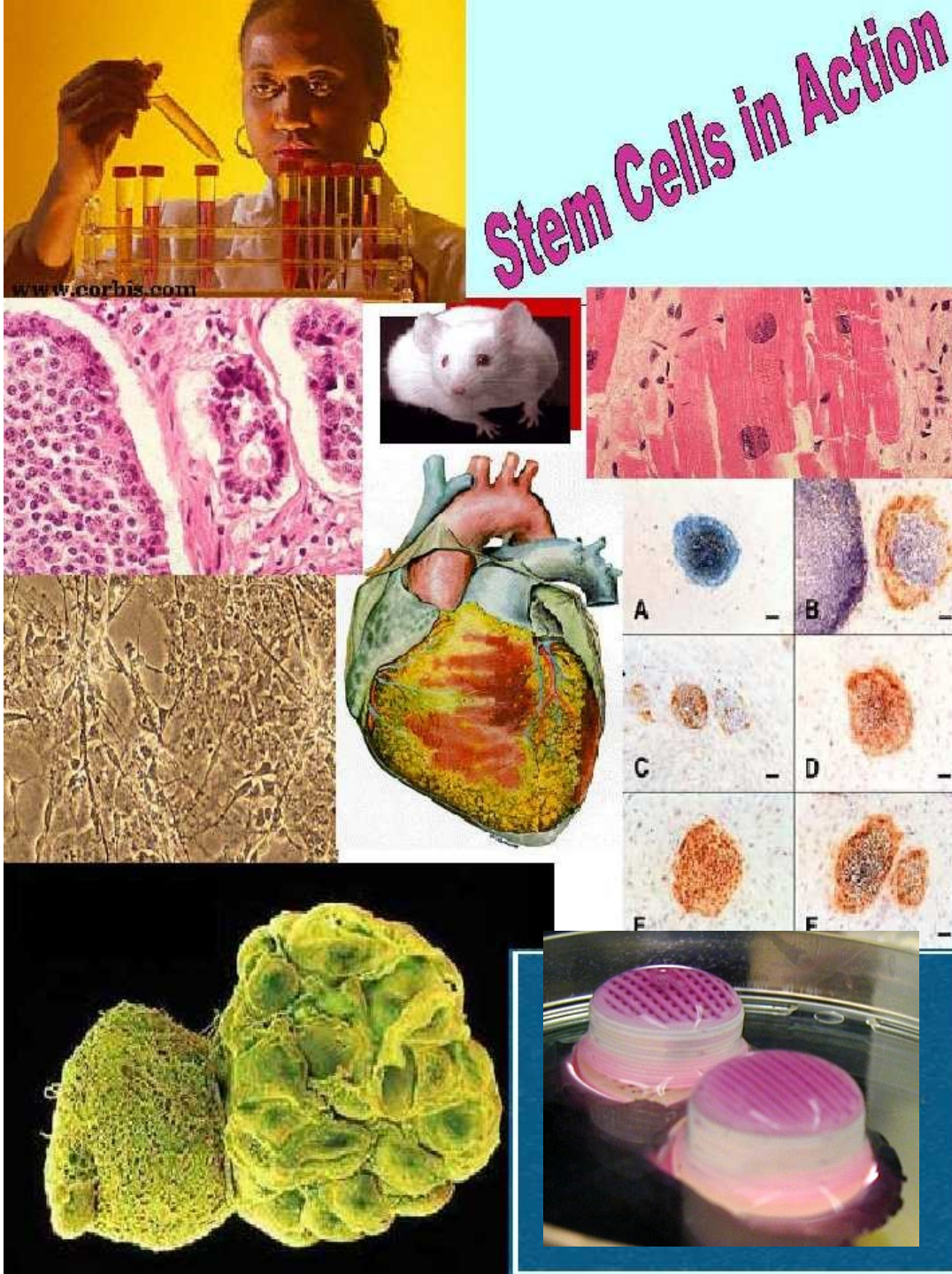
Stem cell application

- Tissue Engineering
- Gene therapy
- Immuo-modulation





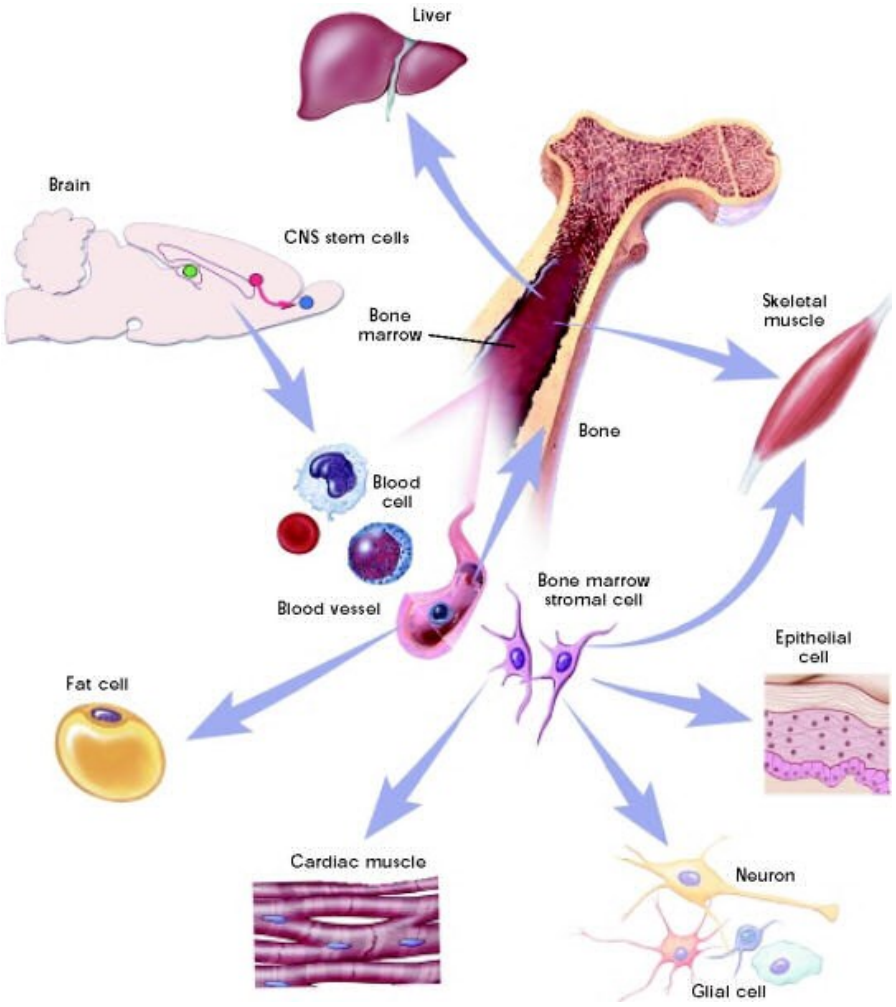
Outline of Discussion

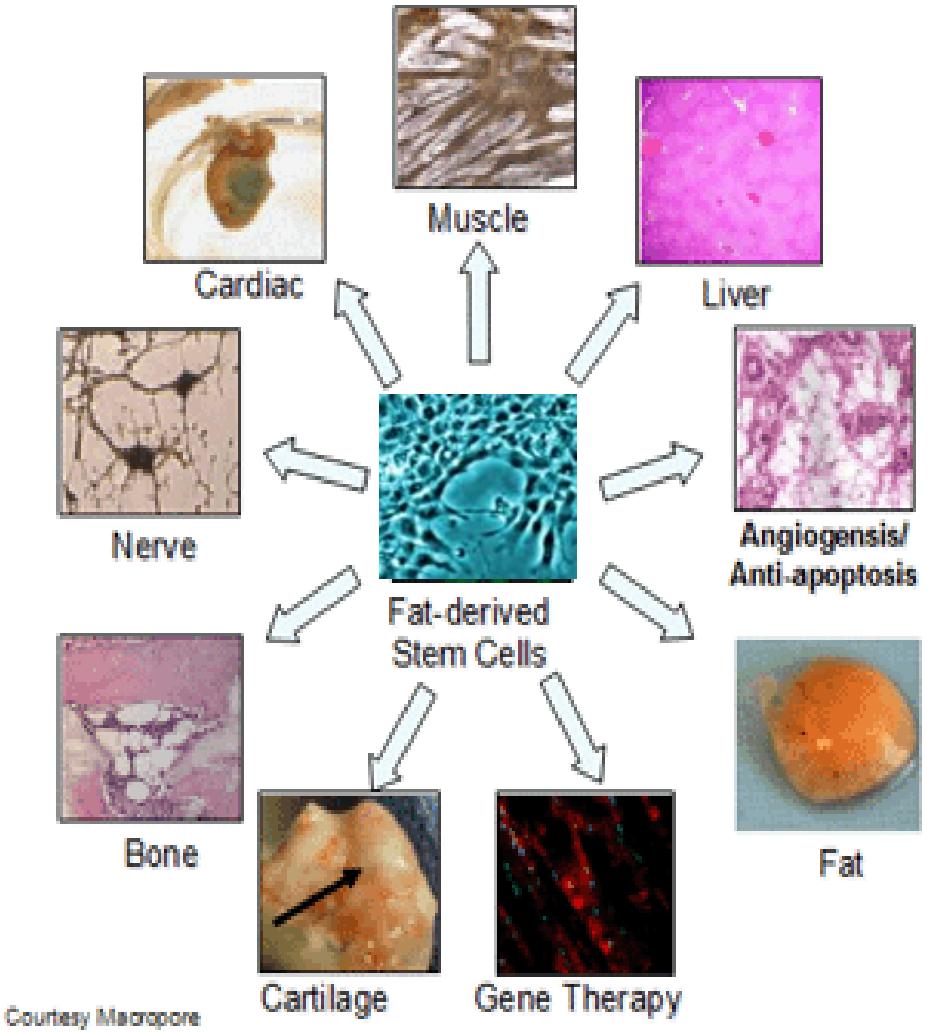
1. Source of stem cells, allogenic MSCs and circulating MSCs.
2. MSCs culture and phenotyping techniques.
3. MSCs systemic recruitment and delivery.
4. Clinical Trials / safety and regulatory issues

Sources of Adult Stem Cells -- MSCs

- Bone marrow
- Adipose tissue
- Periosteum
- Skeletal muscle
- Umbilical cord blood
- Adult peripheral blood
- Bone
- Amniotic fluid
- Organs
- Skin
- Vessels
- Tendon

Bone marrow and Adipose tissue contain multi-potent MSCs





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]

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

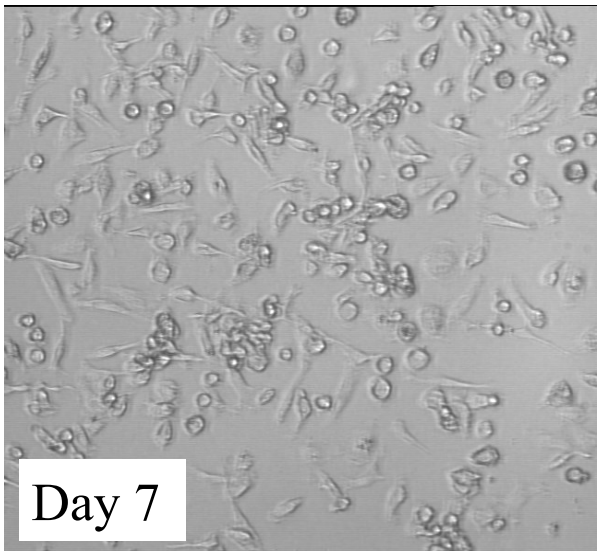
ABSTRACT

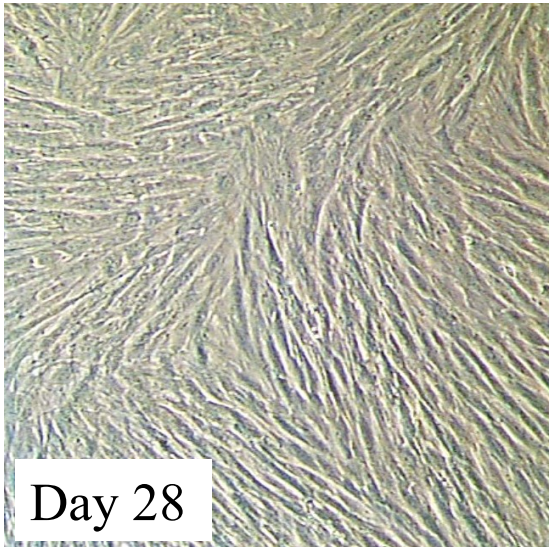
Peripheral blood-derived multipotent mesenchymal stromal cells circulate in low number. They share, most although not all, of the surface markers with bone marrow-derived multipotent mesenchymal stromal cells, possess diverse and complicated gene expression characteristics, and are capable of differentiating 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*

➢ *In search of blood borne MSCs*

Normal Adult Peripheral Blood

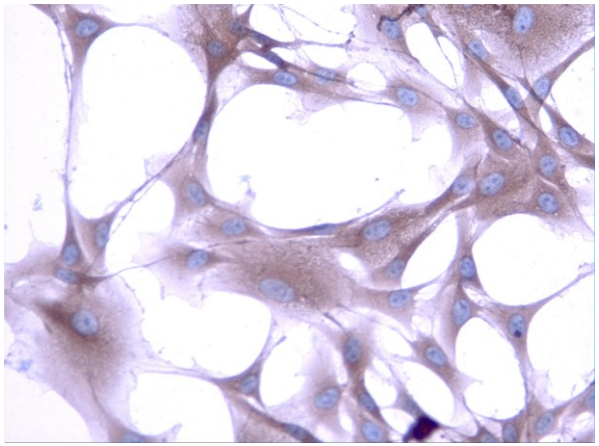
- **1 MSC in ~ 2x10^9 MNCs**
- **(*vs.* 1 MSC in 10^6 bone marrow) MNCs**
- **Numbers of MSCs increased in patients with fracture**



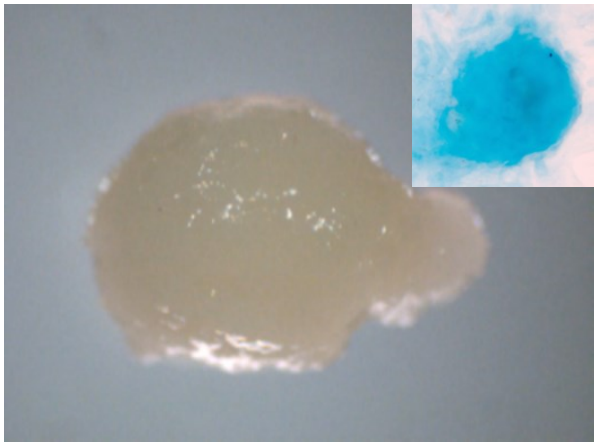


Greater numbers of spindle/polygonal cells fund in the peripheral blood MNCs from the patients with fracture non-union, suggesting a systemic recruitment of MSCs may exist (*Shirley, et al. J. Orthop. Res. 2005: 23 (5): 1013-21*)

Multipotent differentiations of human circulating MSCs

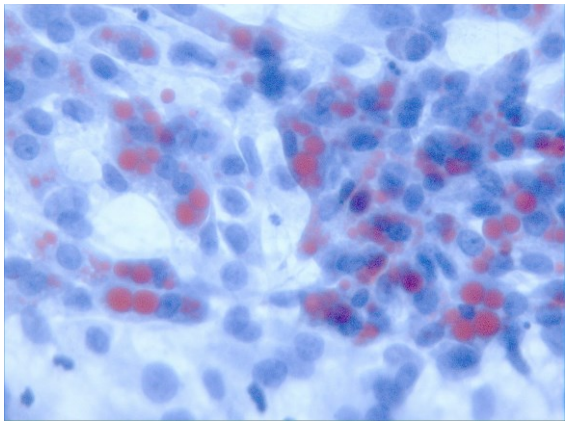


Osteocalcin d21 ×200

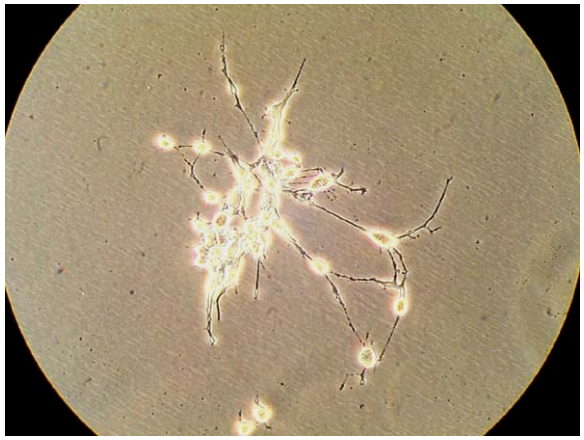


Cell pellet (Cartilage) culture ×4

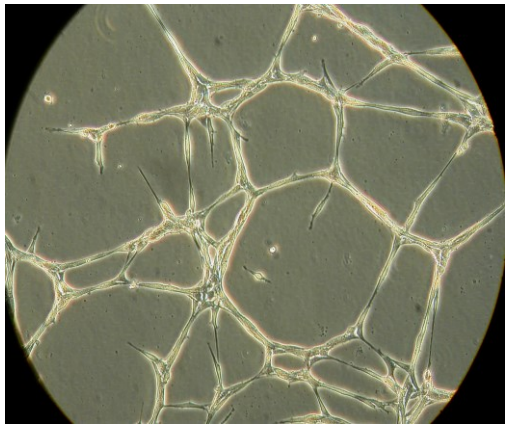
Neurofilament β-ME 6h ×200



Oil red O d21 ×400



β-ME 6h ×100



Long term 2D
culture 72h ×100

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

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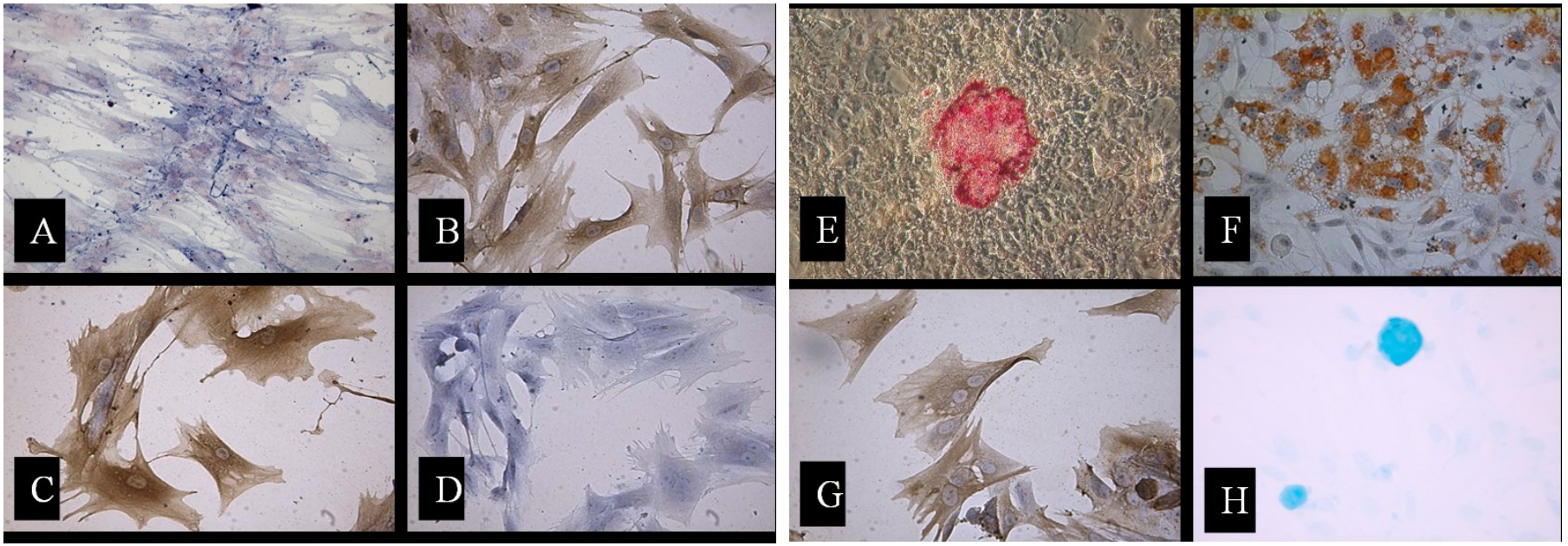
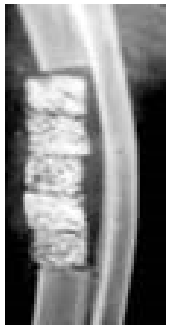
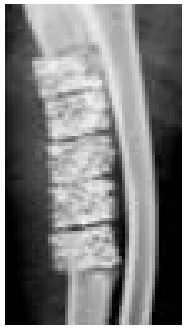
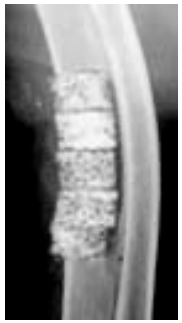
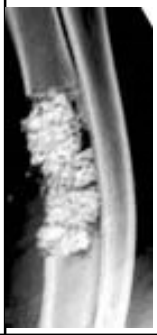
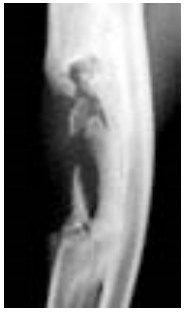
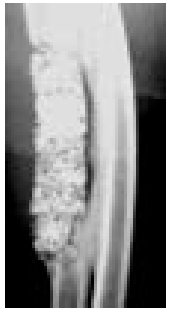
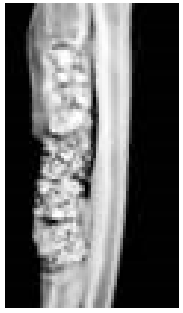
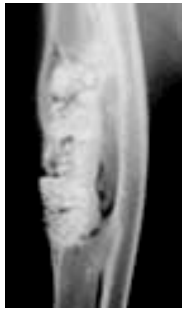
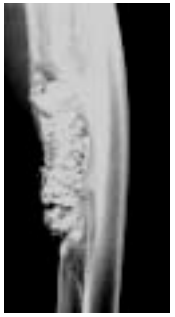
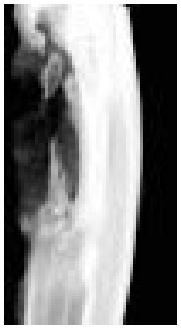
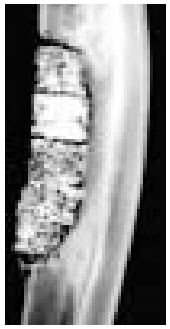
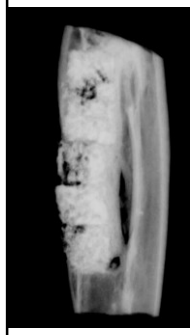
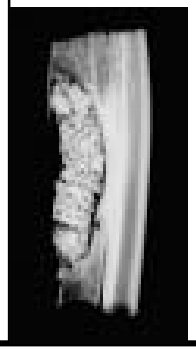
School of Biomedical Sciences, Queen's University Belfast, Musgrave Park Hospital, Belfast, BT9 7JB, United Kingdom



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.

Immunology and Cell Biology (2006) **84**, 413–421 doi:10.1111/j.1440-1711.2006.01458.x

Review Article

Mesenchymal stem cells in immunoregulation

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Summary Mesenchymal stem cells are present within the bone marrow cavity and serve as a reservoir for the continuous renewal of various mesenchymal tissues. Recent studies suggest that mesenchymal stem cells modulate immune reactions *in vitro* and escape from immune surveillance *in vivo*. We provide herein a discussion of issues including the current research progress on the *in vitro* interactions of mesenchymal stem cells with multiple subsets of immune cells (dendritic cells, T cells, B cells and NK cells), *in vivo* transplantation outcomes, the possible underlying mechanisms, future research directions as well as potential clinical implications.

Key words: dendritic cell, immunoregulation, immunosuppression, mesenchymal stem cell, T cell.

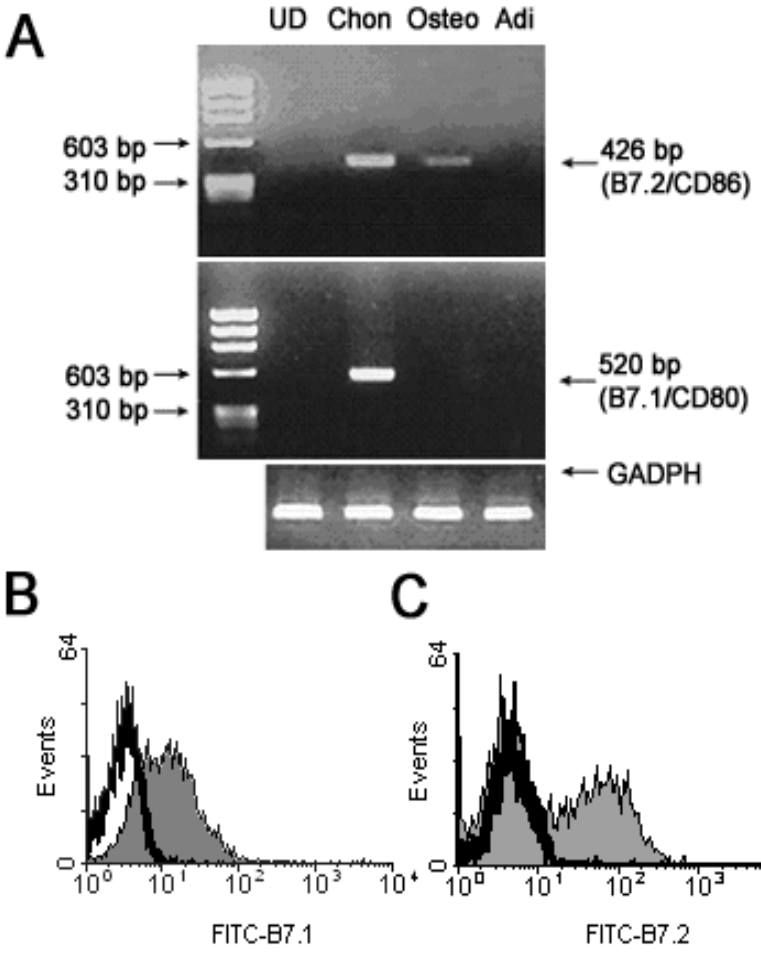
Possible Underlying Mechanisms of MSC Immuno-suppression

- **MSCs may express lower levels HLA-1 and II (human leucocyte antigen class I and II ,they are crucial for triggering immune responses).**

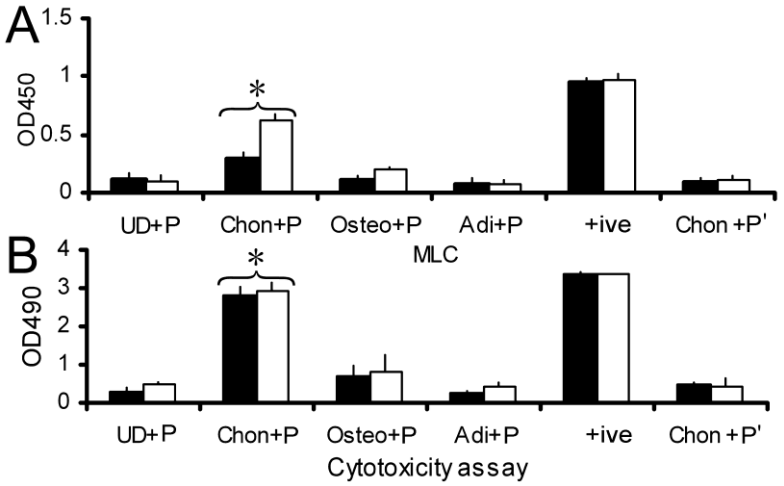
- **MSCs do not express the other co-stimulatory molecules needed for triggering immune responses, such as CD80, CD86, CD40 or CD40L.**

- **MSCs share cell-surface markers with the thymic epithelium such as adhesion molecules, where T cells develop. This may enable MSCs escape the immune surveillance.**

Hypo-immunogenicity of allogenic MSCs – MSCs are immuno-suppresive



RT-PCR data showed that only chondrogenic-differentiated MSCs were positive for both B7 molecules (A). Flow cytometry data showing surface expression of B7 molecules in chondrogenic-differentiated MSCs with corresponding antibodies to B7.1 (B) and B7.2 (C).

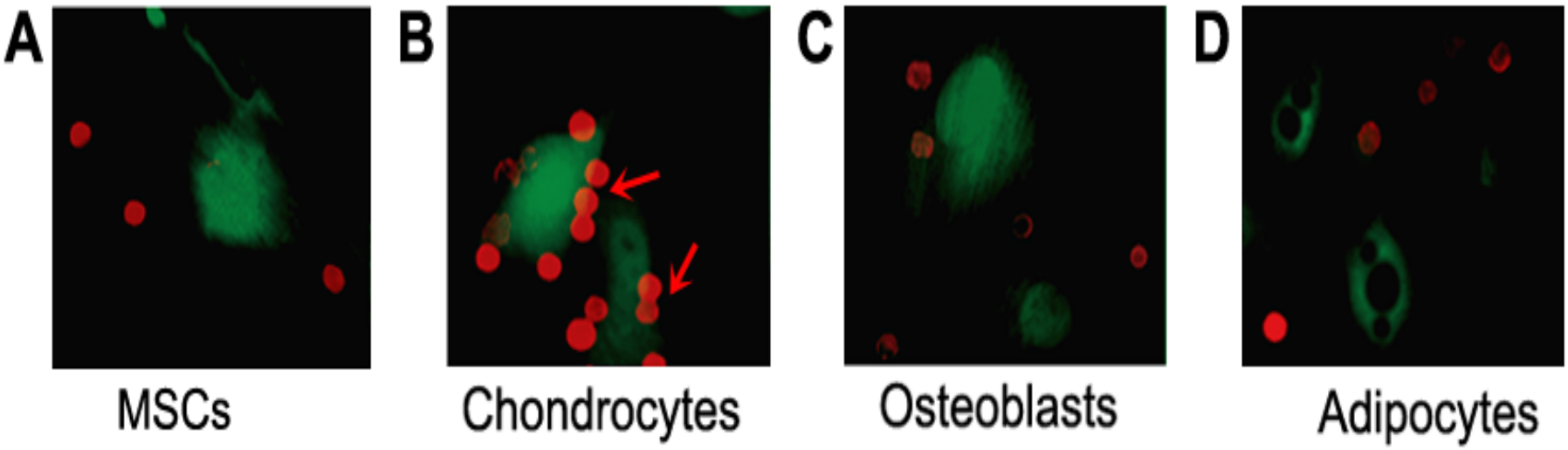


Chondrogenic-differentiated (Chon) not undifferentiated (UD), osteogenic-(Osteo) and adipogenic-(Adi) MSCs, cause proliferation of hPBLs in the presence of hDCs only.

Selected publications:

Chen X, Armstrong, MA, **Li G**. Mesenchymal stem cells in immunoregulation. ***Immunology and Cell Biology***; 2006; 84(5): 413-421.

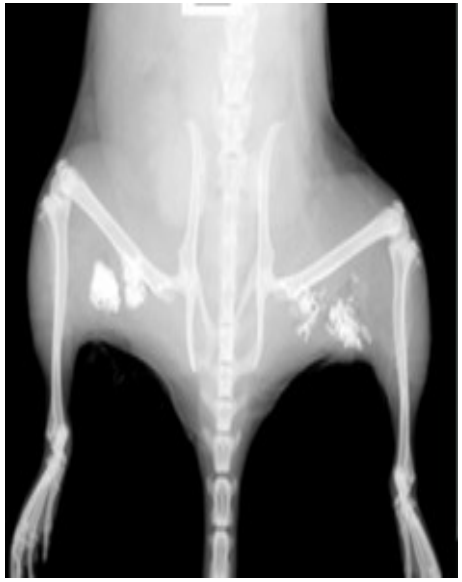
Chen X, McClurg A, Zhou GQ, McCaiguea M, Armstrong MA, **Li G**. Chondrogenic-differentiation rectifies the immunosuppressive property of bone marrow-derived MSCs and the effect is partially due to the up-regulated expression of B7 molecules. ***Stem Cells***; 2007; 25: 364-370.



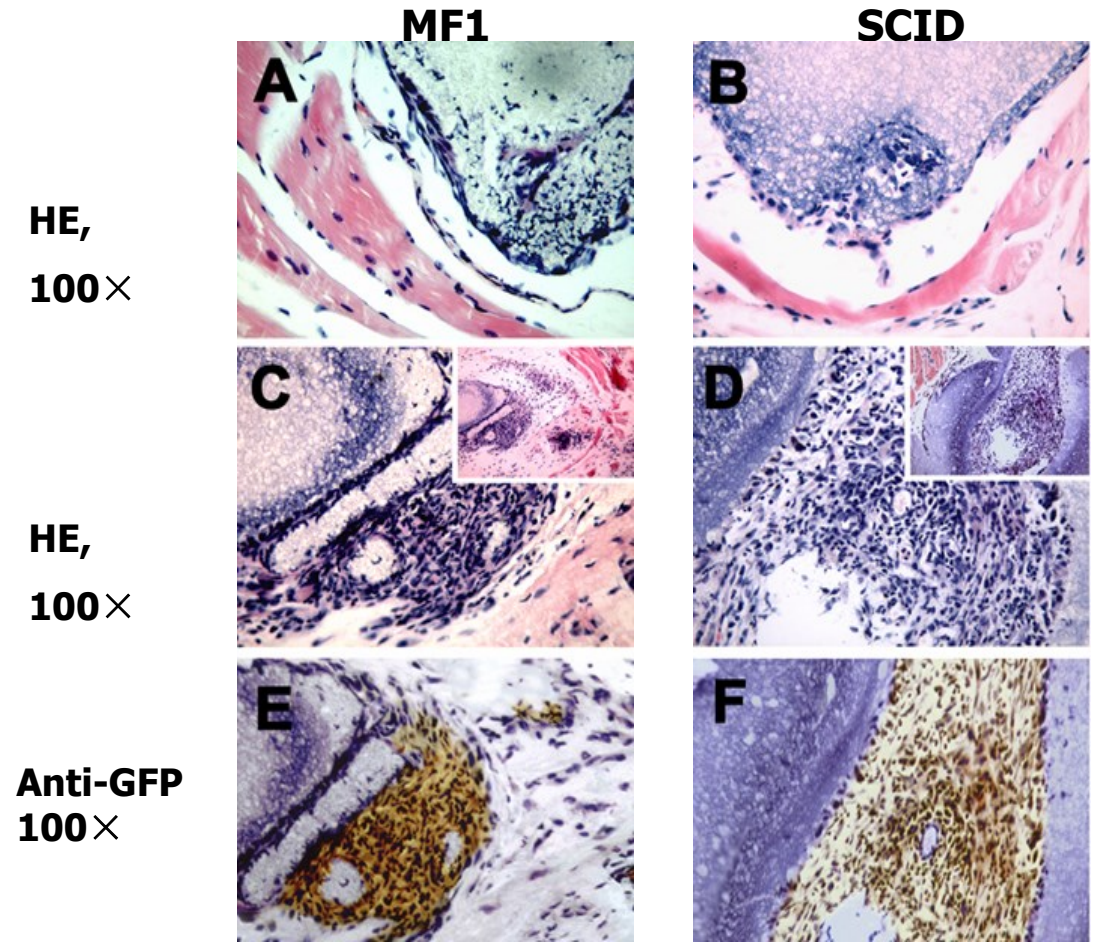
Chondrogenic-differentiated MSCs are capable of rosetting (binding) hDCs. PKH-26-labeled (red) hDCs were co-cultured with GFP undifferentiated, chondrogenic-, osteogenic- and adipogenic-differentiated MSCs at 37ºC for 3 days. hDCs formed rosette-rings around co-cultured chondrogenic-differentiated MSCs (B, red arrow), whereas only sparsely distributed hDCs were seen in other groups (A,C,D). The binding rate of chondrogenic-differentiated MSCs was 45%, and 4-7% for the other groups.

Chen X, et al. Stem Cells; 2007; 25:364-370

Xenogenic MSCs survived cell implantation for 12 weeks



- GFP rat MSCs
- Implanted into MF1 and SCID mice for 12 weeks

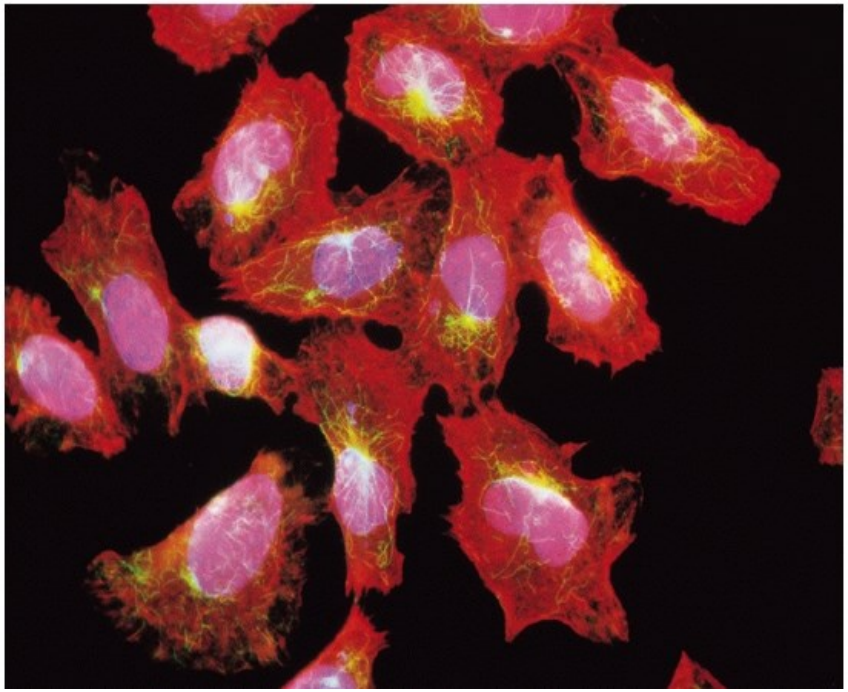


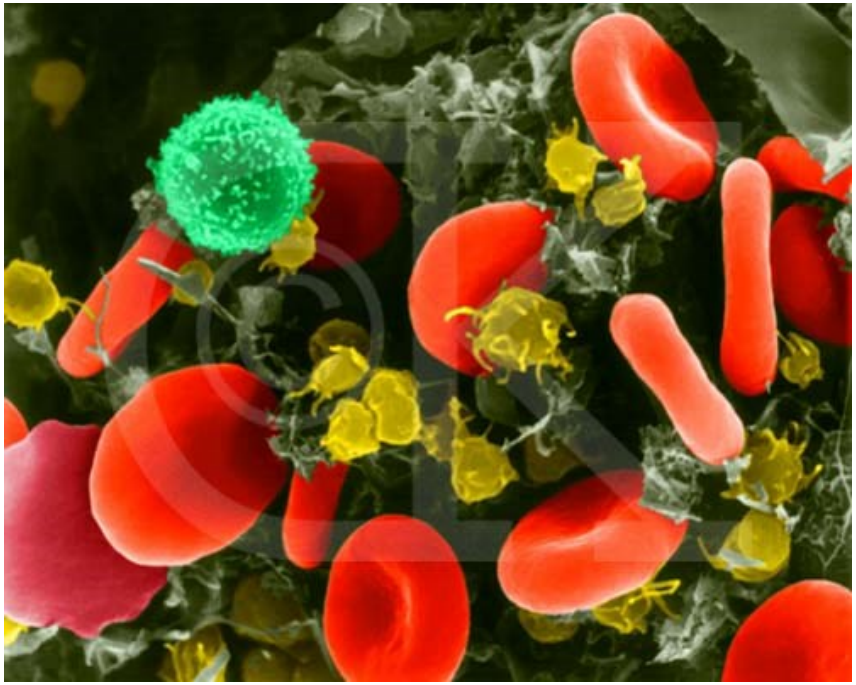
Wang Y, Chen X, Armstrong M, Li G. "Survival of xenogeneic bone marrow-derived mesenchymal stem cells in a xeno-transplantation model." Journal of Orthopaedic Research; 2007; 25: 926-932.

Outline of Discussion

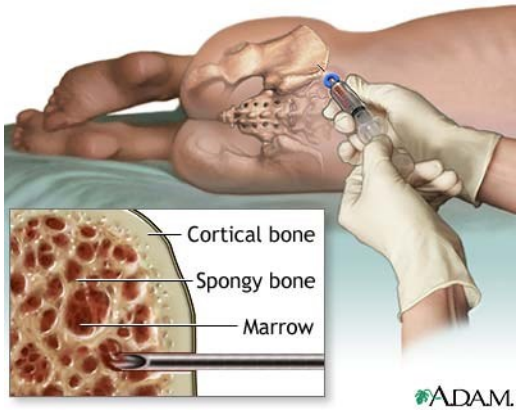
1. Source of stem cells, allogenic MSCs and circulating MSCs.
2. MSCs culture and phenotyping techniques.
3. MSCs systemic recruitment and delivery.
4. Clinical Trials / safety and regulatory issues

Mesenchymal stem cells (MSCs)





BMMNCs Isolation



bone marrow

density gradient centrifugation

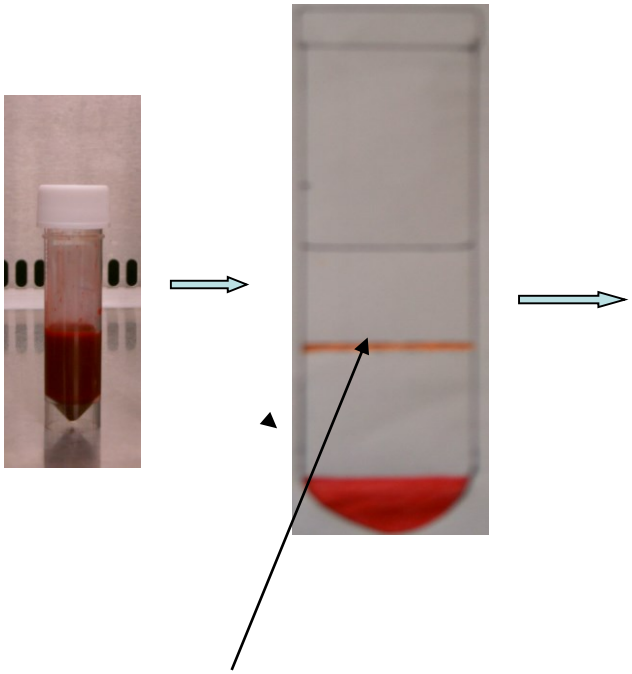
over Lymphoprep

BMMNCs

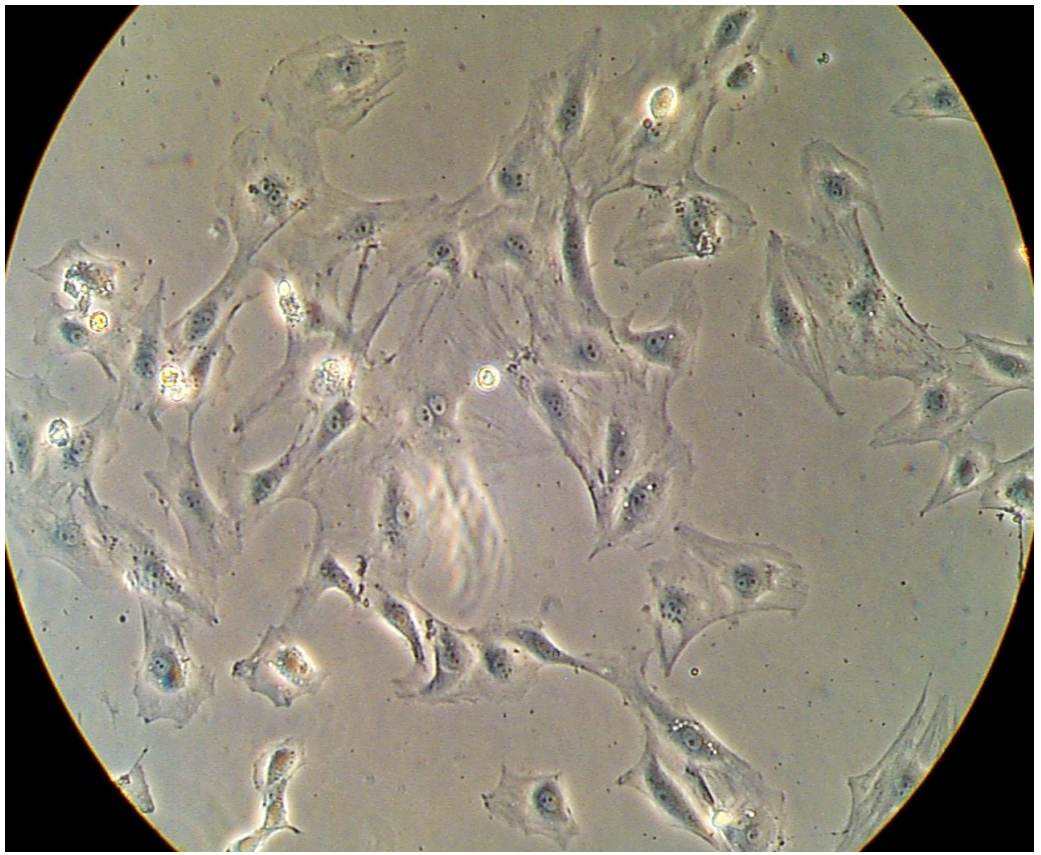
PBS wash

BMMNCs culture

BM-derived CFU-F

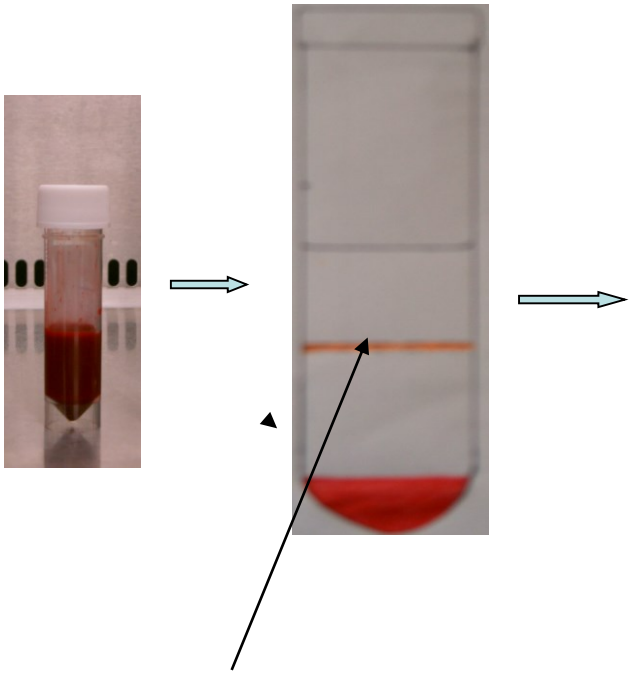


Mononuclear cell layer

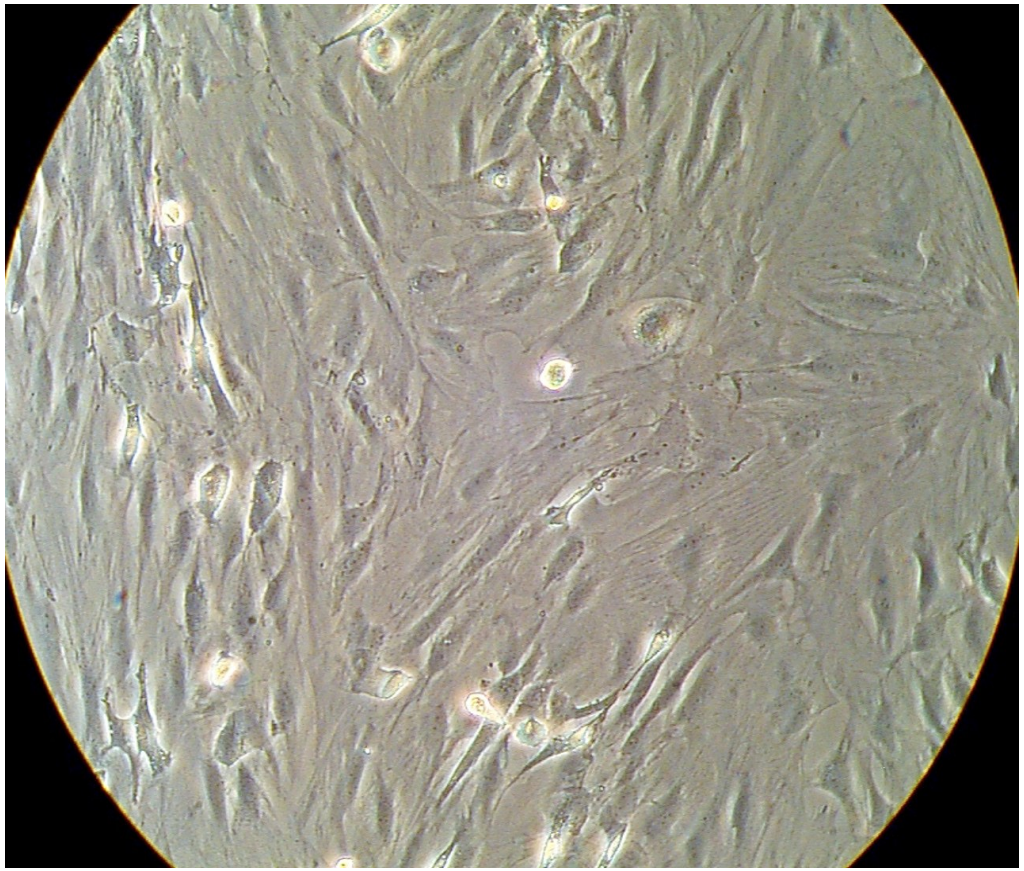


12-14 days

BM-derived CFU-F

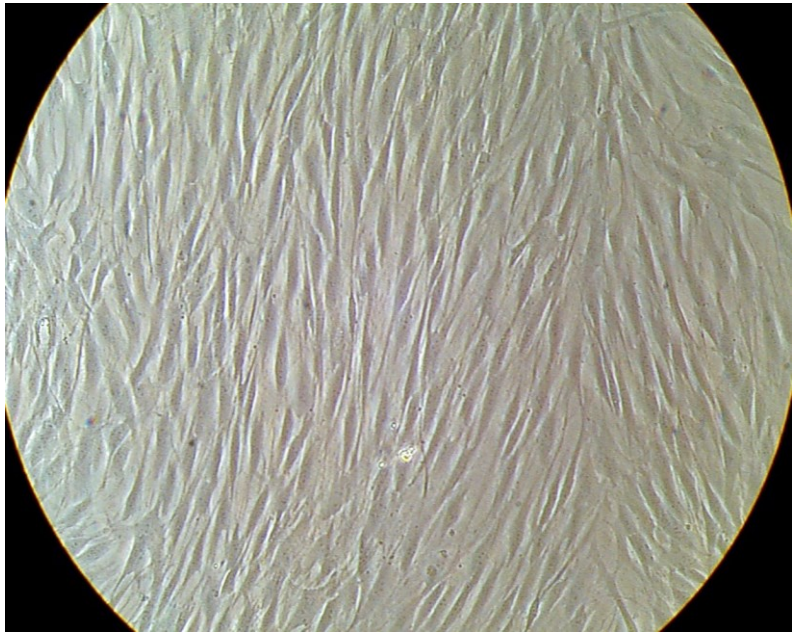


Mononuclear cell layer



At confluence

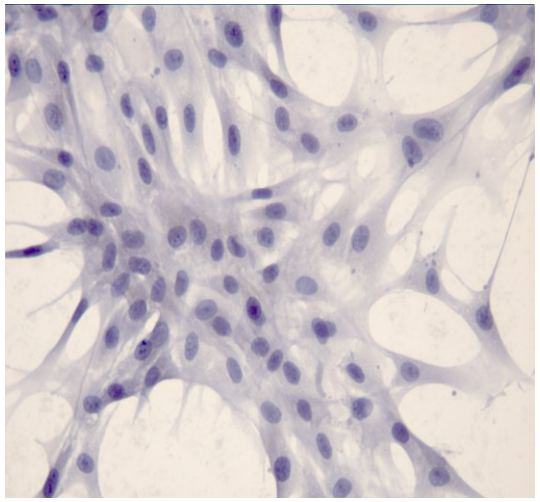
BM-derived CFU-Fs

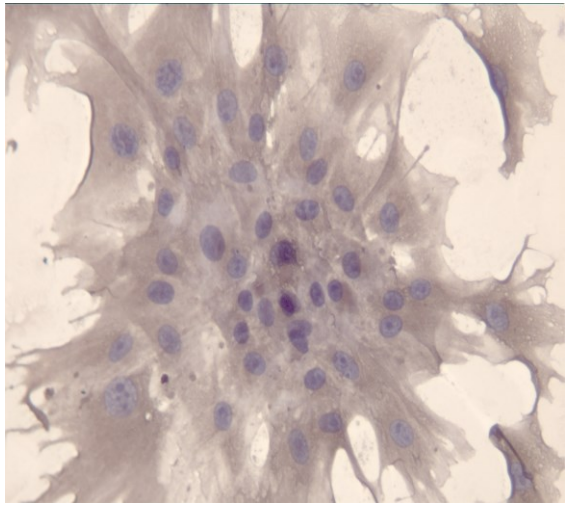


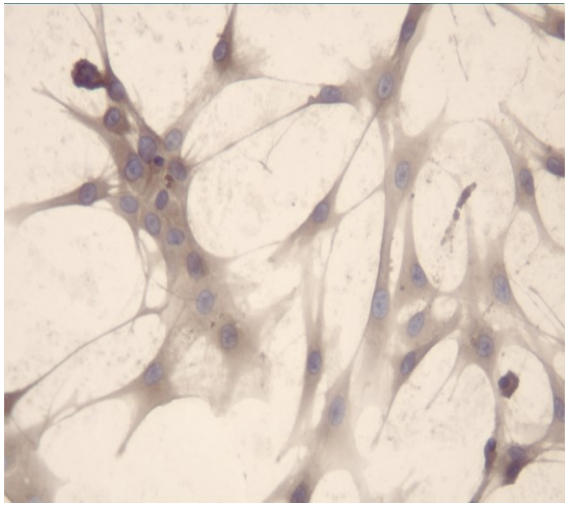
CFU-F colony

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

BM





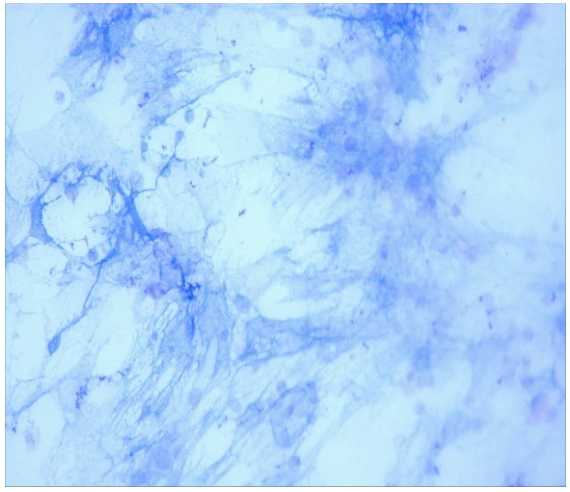


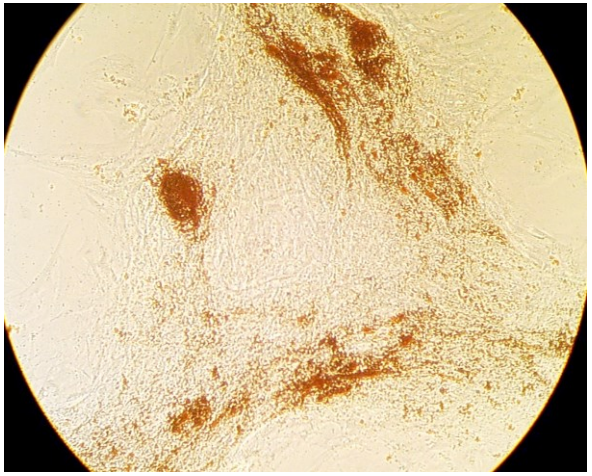
Negative control

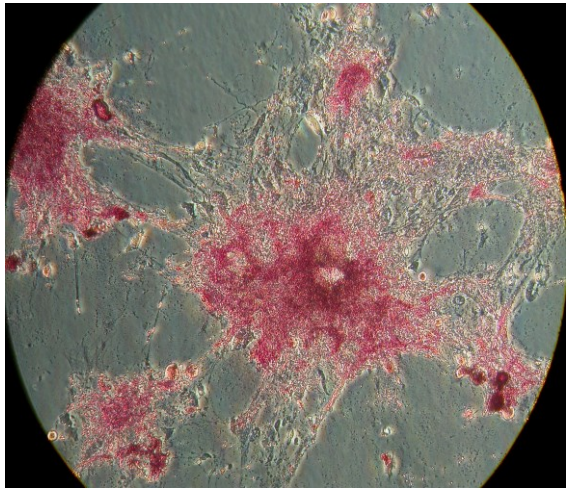
Type Ⅰ collagen

Osteocalcin

BM







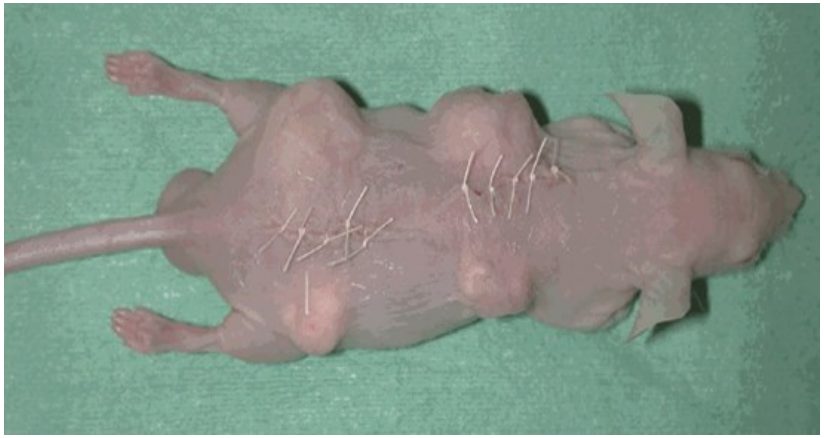
ALP

Von Kossa

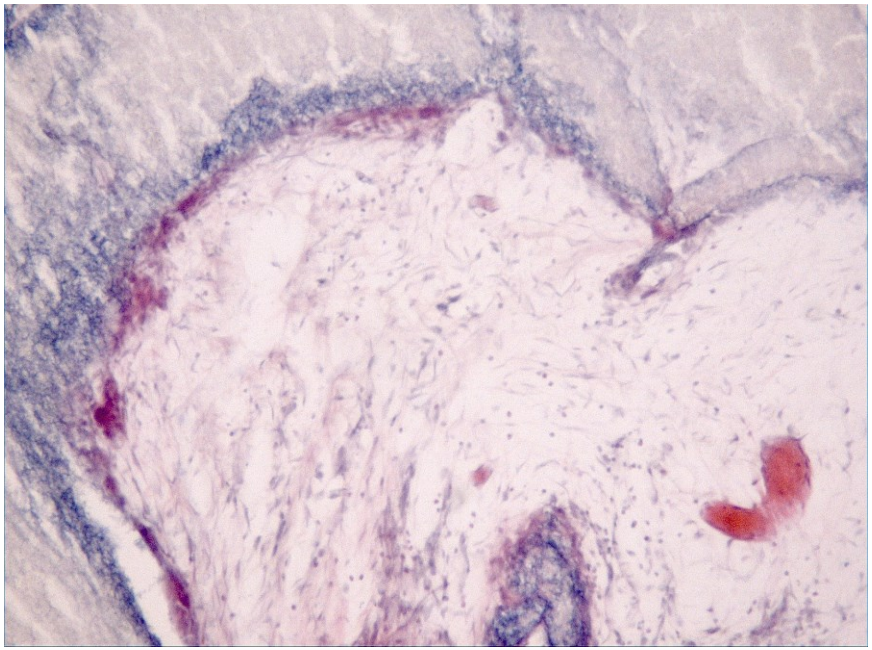
Alizarin red S

In-Vivo Transplantation Assay

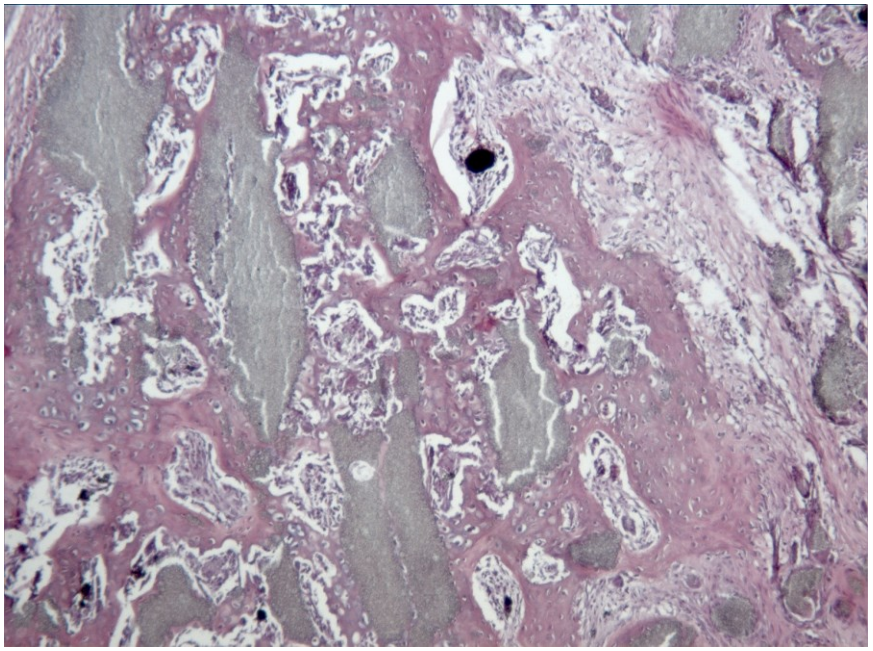
- BM stromal cells on passage 6
- Cells were seeded into scaffolds (Absorbable porous calcium phosphate substitute)
- Scaffolds loaded with cells were implanted subcutaneously onto the back of SCID mice for 12 weeks



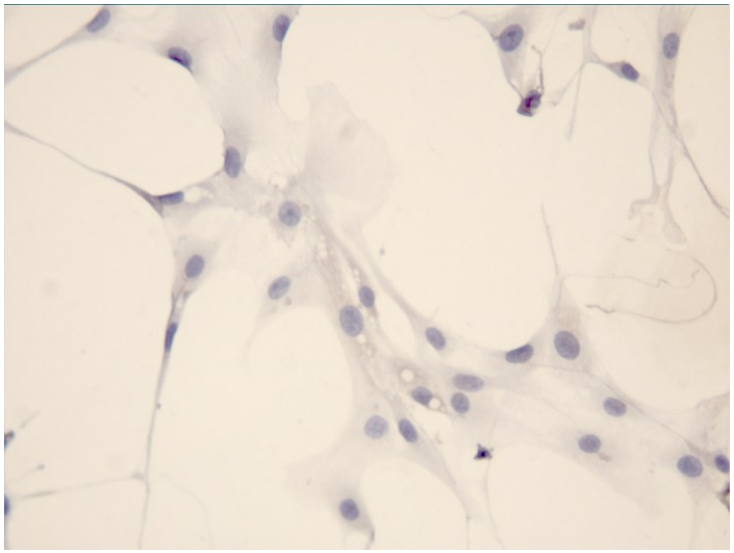
H&E Staining



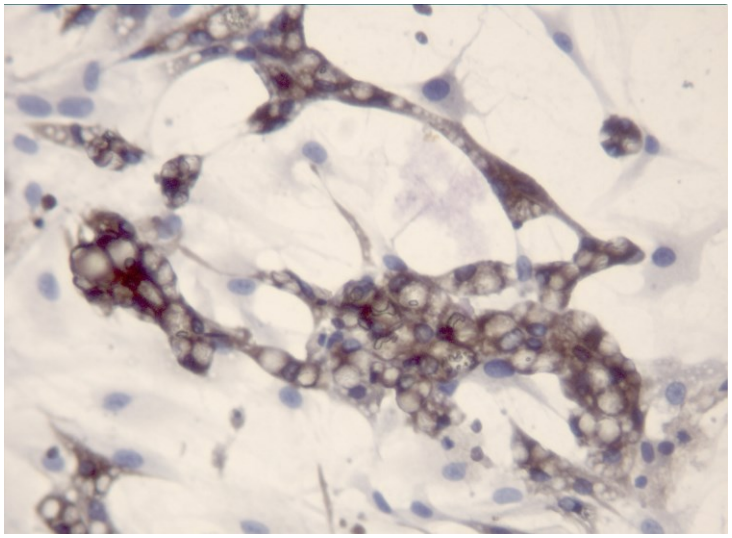
Biomaterials without BM cells



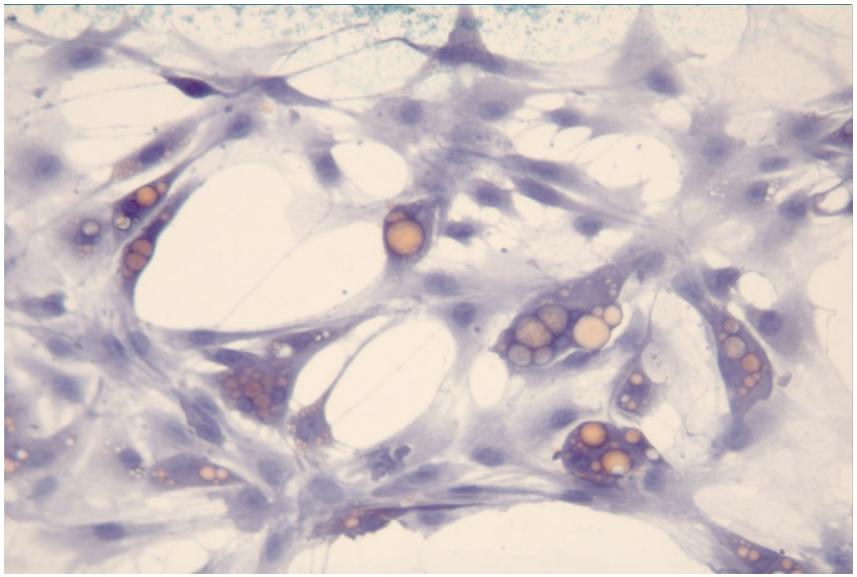
Biomaterials with BM cells



Negative control

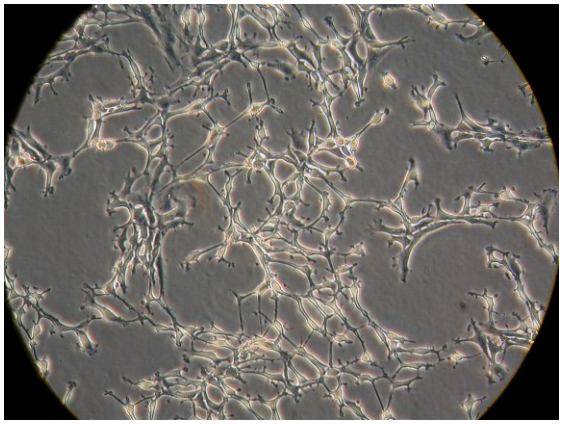


C/EBP α

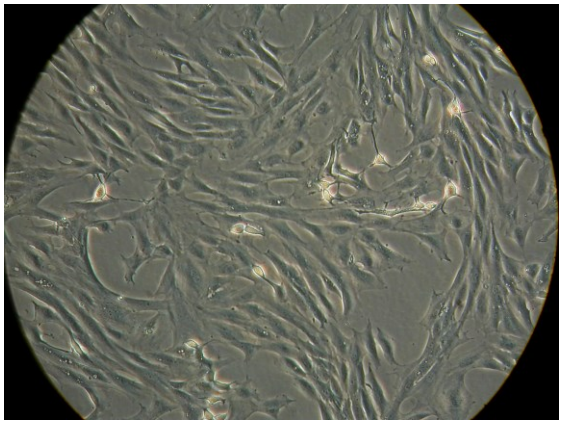


Oil Red O

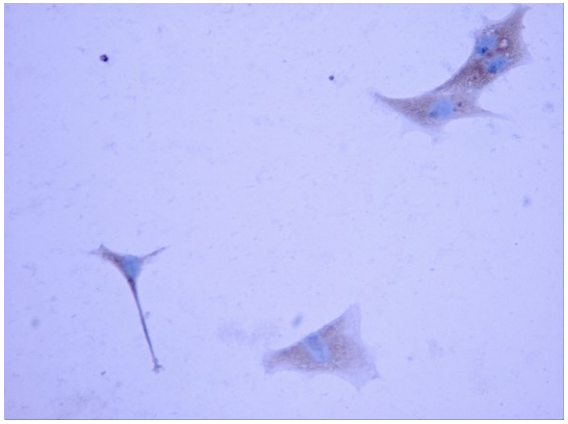
Neurogenic differentiaiton



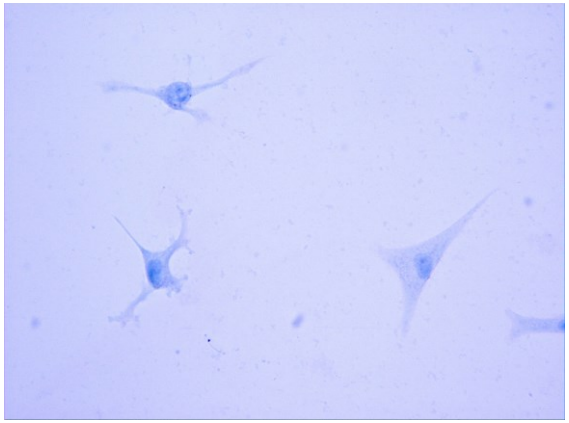
β-ME 6h ×100



Control ×100



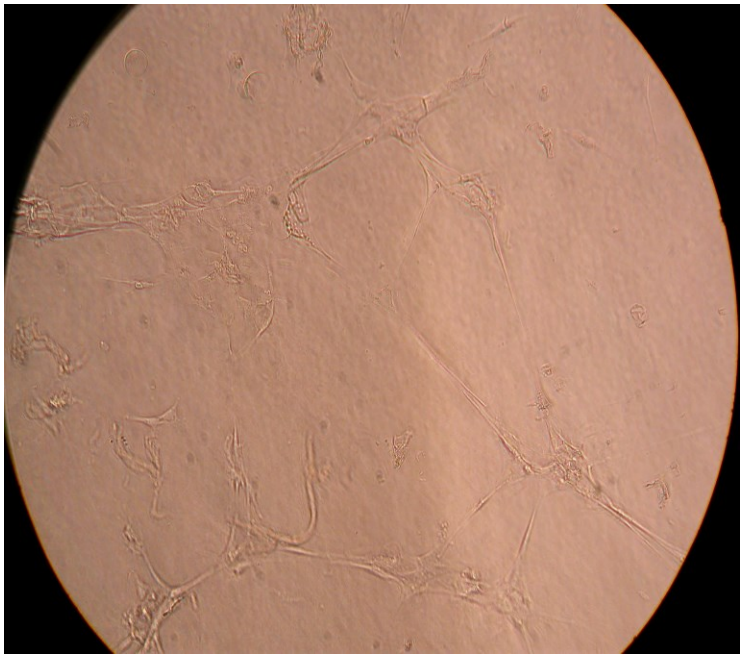
Neurofilament β-ME 6h ×200



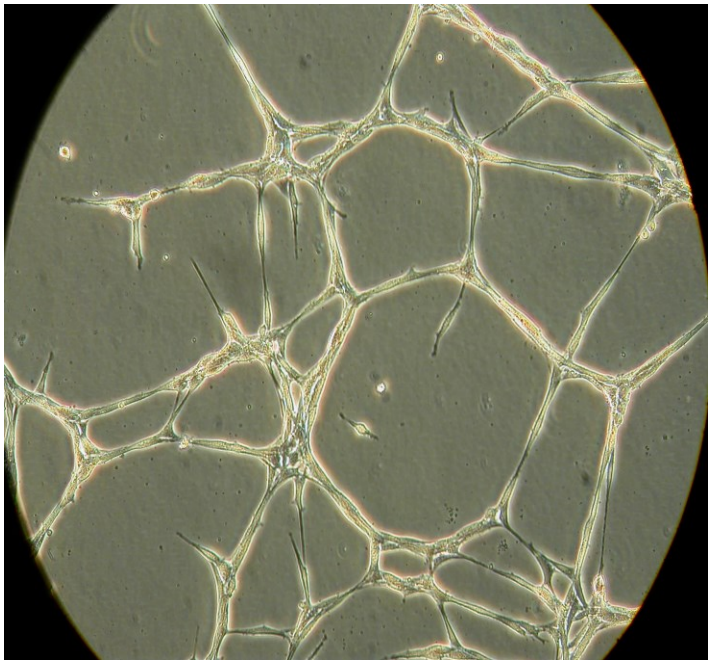
Control ×200

Differentiation Potential of MSCs

In vitro angiogenesis

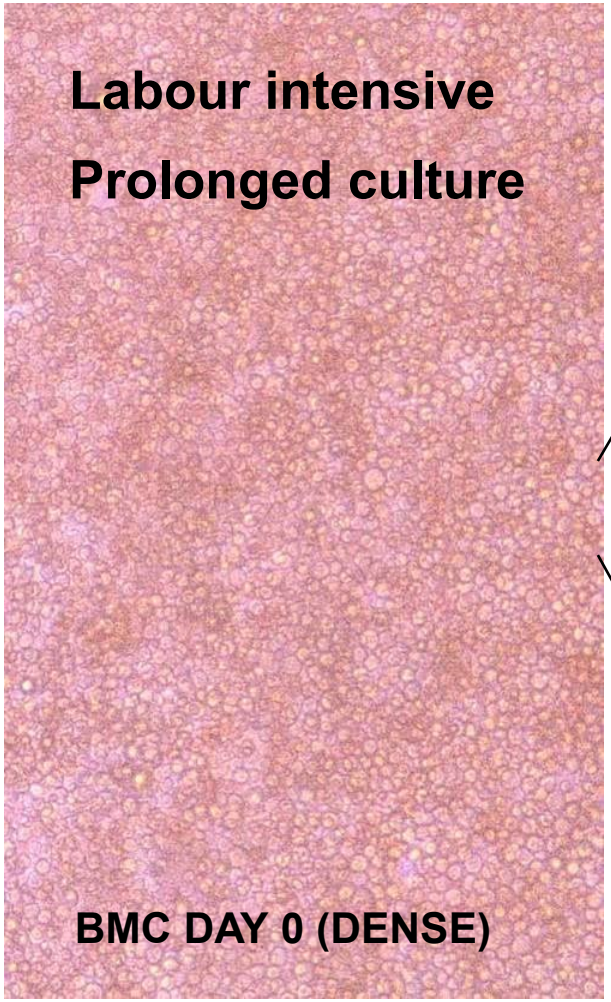


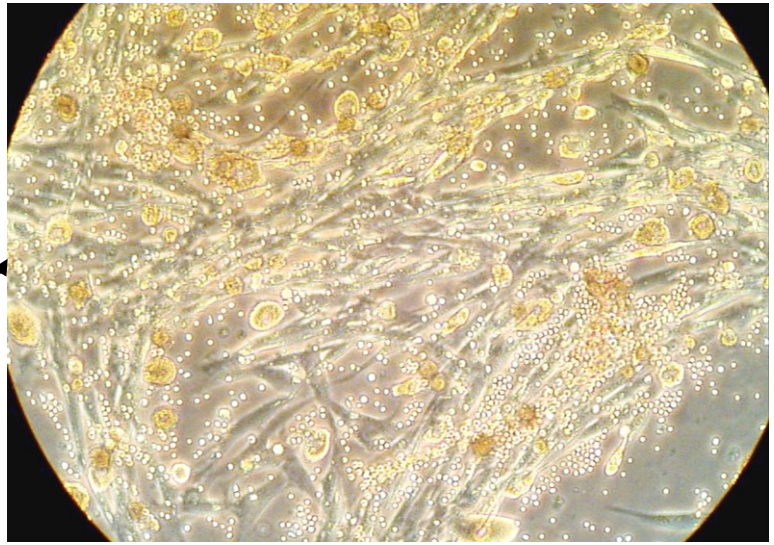
Matrigel 3D culture 24h
×100

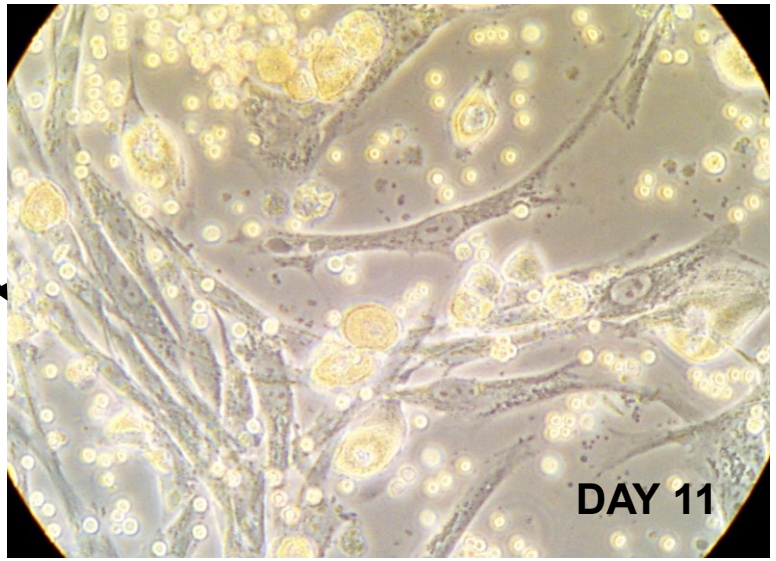


Long term 2D culture 72h
×100

Bone Marrow Derived MSCs Culture







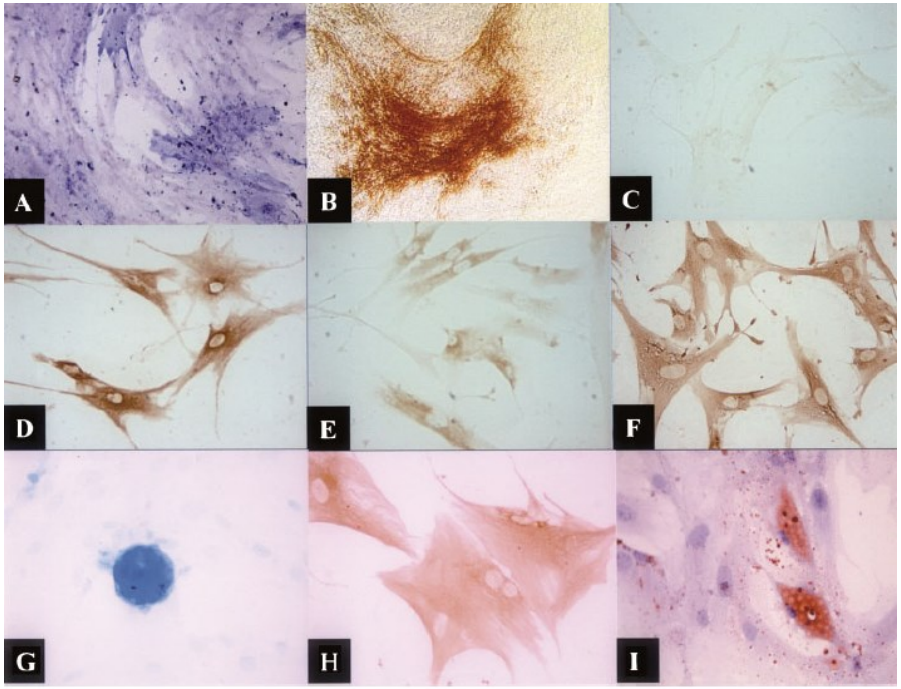
Plating density – 500-1000/cm2

Nonadherent Cell Population of Human Marrow Culture Is a Complementary Source of Mesenchymal Stem Cells (MSCs)

Chao Wan, Qiling He, Mervyn McCaigue, David Marsh, Gang Li

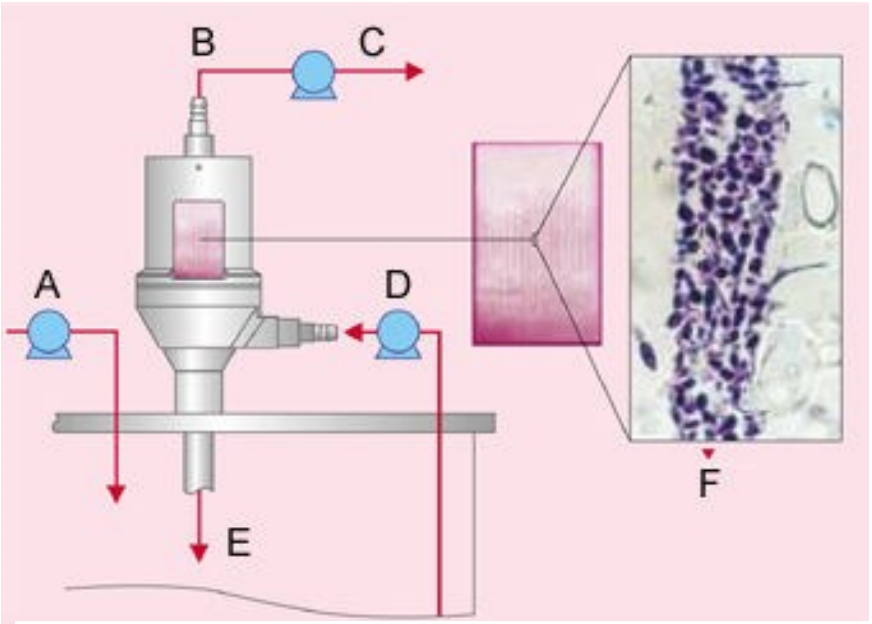
Department of Trauma and Orthopaedic Surgery, Institute of Medical Research, Queen's University Belfast, Musgrave Park Hospital, Belfast BT9 7JB, United Kingdom

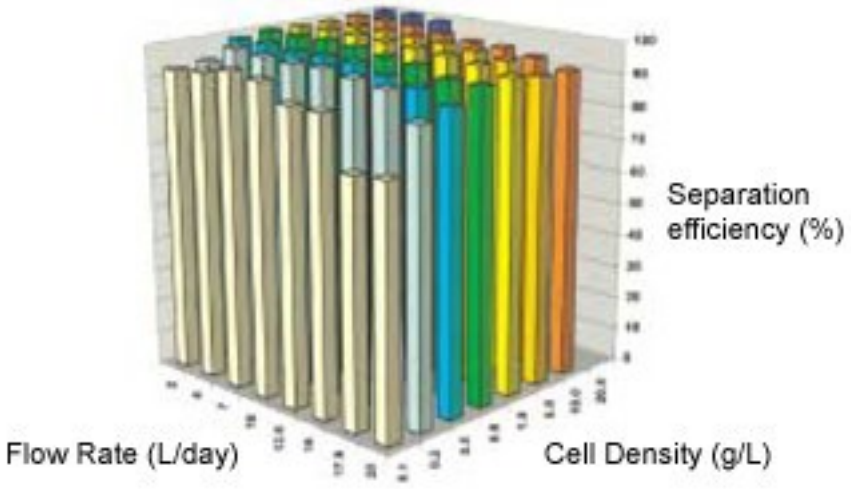
In conclusion, this study established a simple and cost-effective method to increase the number of MSCs by replating the nonadherent cell population in the bone marrow cell cultures. The nonadherent MSCs maintained similar osteognic potentials in vitro and in vivo as the adherent MSCs do, and they may serve as a complementary source of MSCs to facilitate the clinical applications of MSCs in tissue engineering and cell therapy.

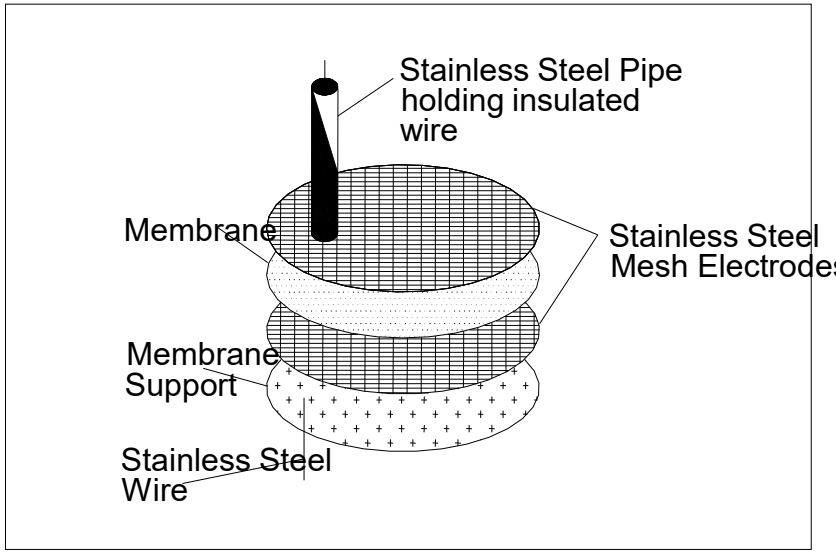


Journal of Orthopaedic Research 2006; 24:21-28.

Perfusion Bioreactors







STEM CELLS®

TECHNOLOGY DEVELOPMENT

Stem Cells 2006; 24:2052-2059.

Bioreactor Expansion of Human Adult Bone Marrow-Derived Mesenchymal Stem Cells

XI CHEN,[a] HAIBO XU,[b] CHAO WAN,[a] MERVYN MCCAIGUE,[a] GANG LI[a]

[a]Department of Orthopaedic Surgery, [b]Department of Surgery, Center for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, United Kingdom

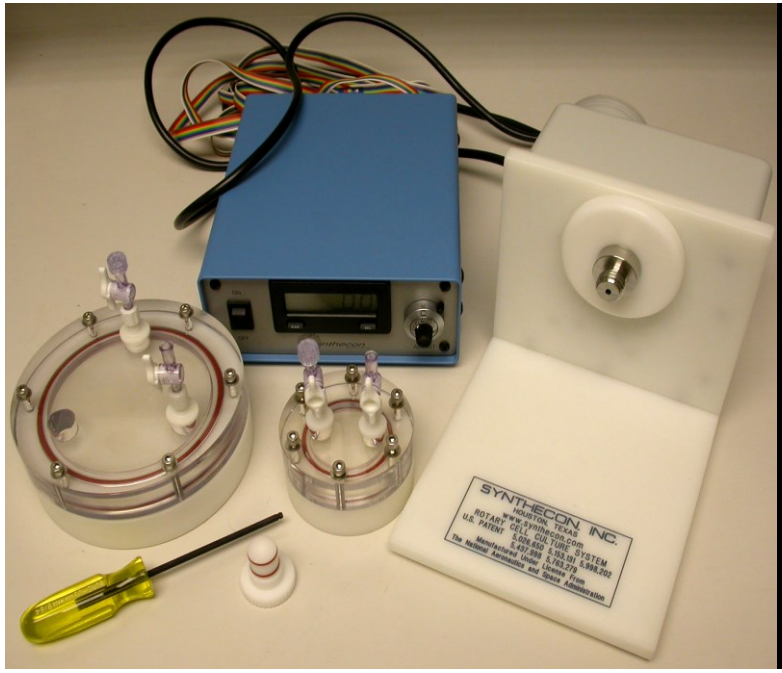
Key Words. Mesenchymal stem cells • Hematopoietic stem cells • Bioreactor • Differentiation

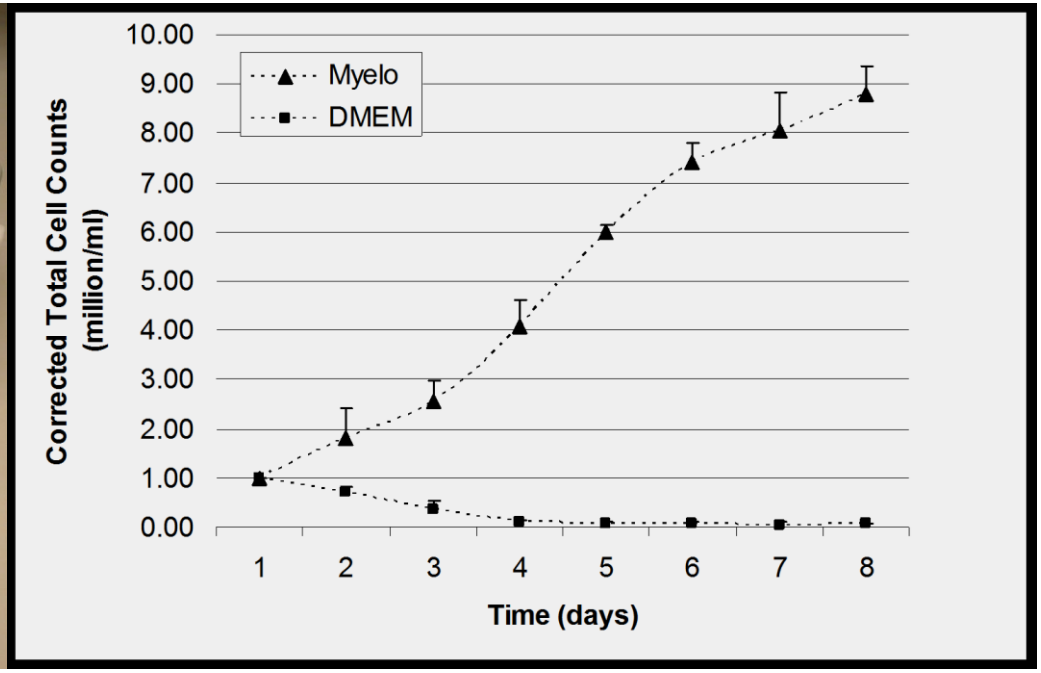
ABSTRACT

Supplementation of mesenchymal stem cells (MSCs) during hematopoietic stem cell (HSC) transplantation alleviates complications such as graft-versus-host disease, leading to a speedy recovery of hematopoiesis. To meet this clinical demand, a fast MSC expansion method is required. In the present study, we examined the feasibility of using a rotary bioreactor system to expand MSCs from isolated bone marrow mononuclear cells. The cells were cultured in a rotary bioreactor with Myelocult medium containing a combination of supplementary factors, including stem cell factor and interleukin-3 and -6. After 8 days of culture, total cell numbers, Stro-1$^+$CD44$^+$CD34$^-$ MSCs, and CD34$^+$CD44$^+$Stro-1$^-$ HSCs were increased 9-, 29-, and 8-fold, respectively. Colony-forming efficiency-fibroblast per day of the bioreactor-treated cells was 1.44-fold higher than that of the cells without bioreactor treatment. The bioreactor-expanded MSCs showed expression of primitive MSC markers endoglin (SH2) and vimentin, whereas markers associated with lineage differentiation, including osteocalcin (osteogenesis), type II collagen (chondrogenesis), and C/EBP-α (CCAAT/enhancer-binding protein-α) (adipogenesis), were not detected. Upon induction, the bioreactor-expanded MSCs were able to differentiate into osteoblasts, chondrocytes, and adipocytes. We conclude that the rotary bioreactor with the modified Myelocult medium reported in this study may be used to rapidly expand MSCs.

STEM CELLS *2006;24:2052–2059*

MSCs can be rapidly expanded in Bioreactor





➢**After 8-day Myelocult™ bioreactor culture, total cell numbers, Stro-1^{+}CD44^{+} MSCs were expanded 9 and 29-fold of starting population.**

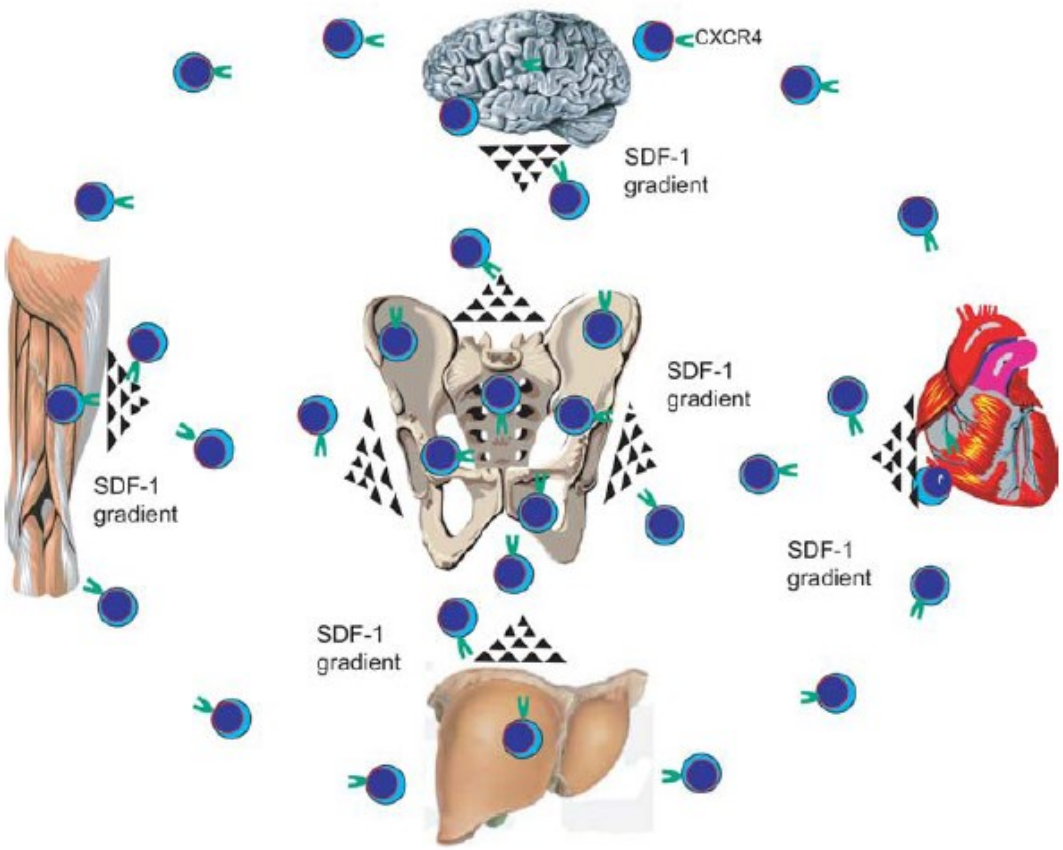
➢**CFE-F assay demonstrated that Myelocult™ bioreactor-expanded MSCs had significantly higher CFE-F/day than static DMEM controls.**

Chen X, Xu H, Wan C, McCaigue M, Li G. "Bioreactor expansion of human adult bone marrow-derived mesenchymal stem cells (MSCs)" Stem Cells; 2006; 24: 2052-2059.

Outline of Discussion

1. Source of stem cells, allogenic MSCs and circulating MSCs.
2. MSCs culture and phenotyping techniques.
3. MSCs systemic recruitment and delivery.
4. Clinical Trials / safety and regulatory issues

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,*]

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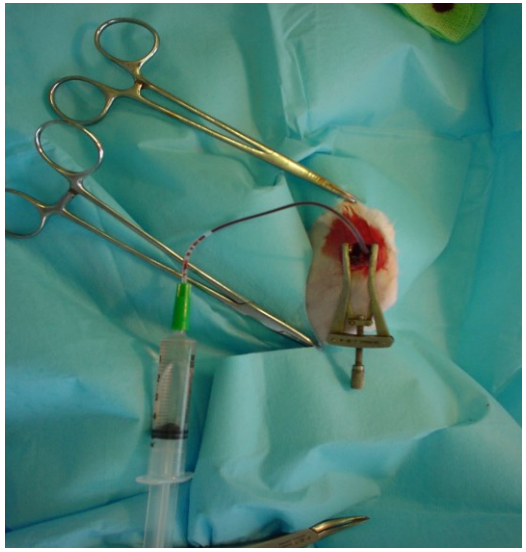
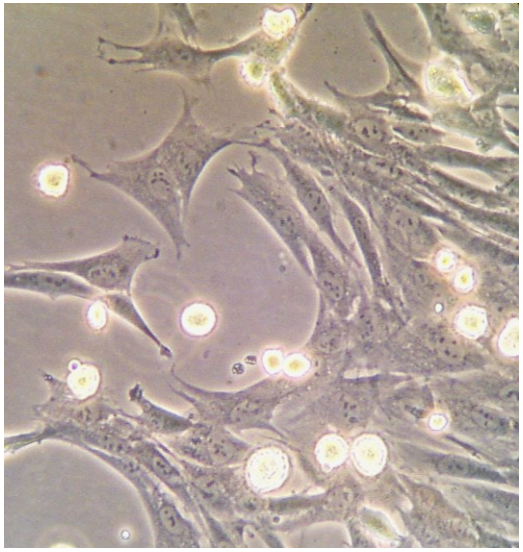
[b] *Department of Pathology, Royal Victoria Hospital, Belfast BT12 6BJ, UK*

Accepted 28 January 2005

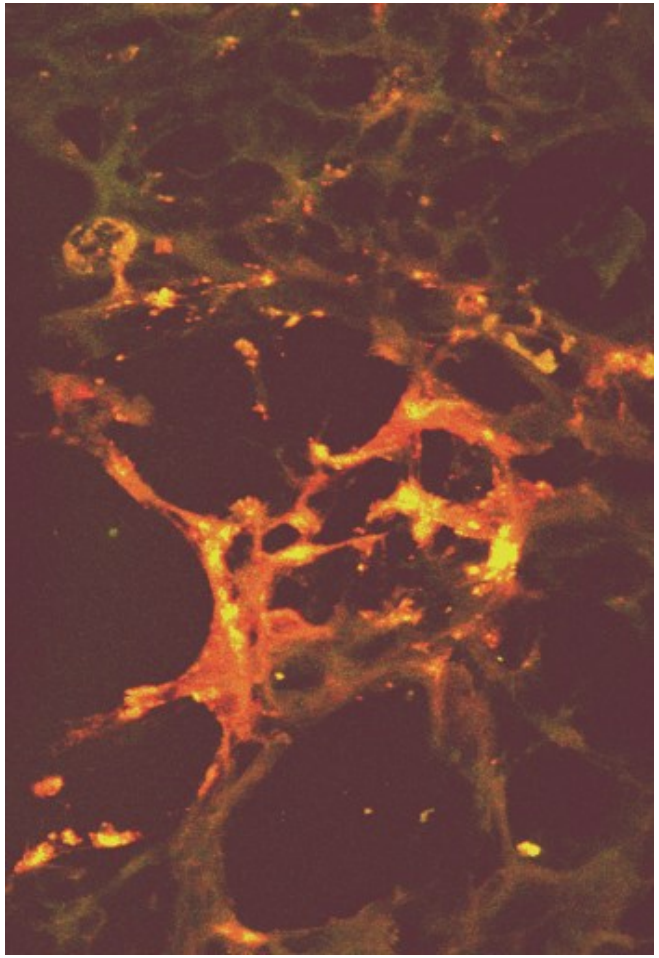
MSCs homes to fracture sites through peripheral circulation

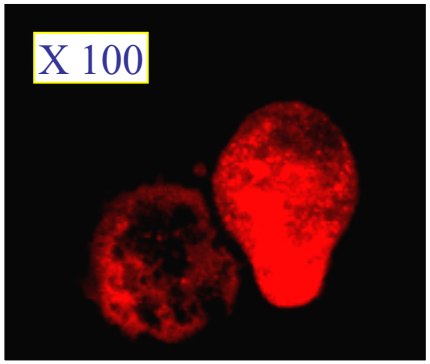


Bone marrow harvested



Rabbit bone marrow
MSCs culture





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

Re-implantation

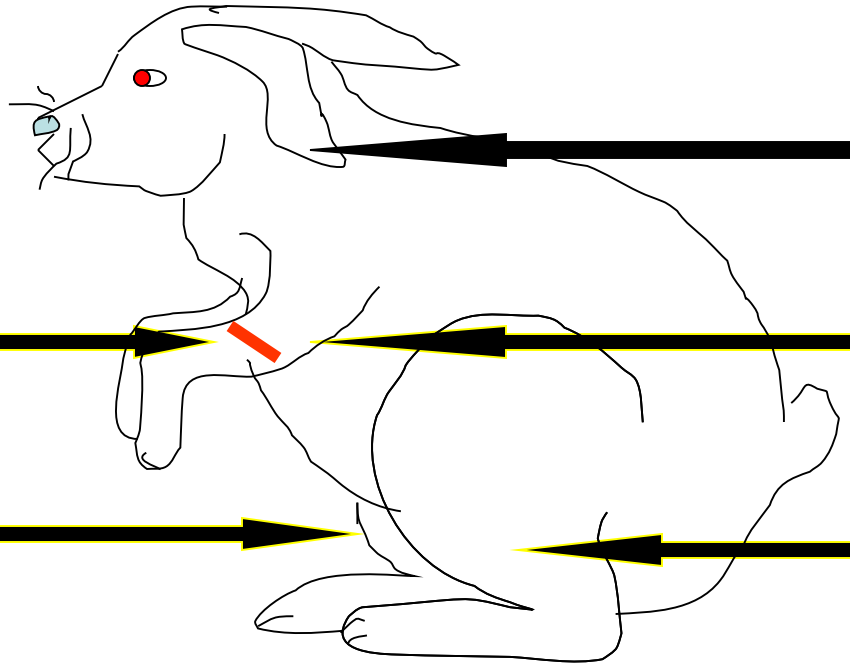
48 hours post Fx

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

Culture 3 weeks

Ulnar defect

BM harvest



Control E

Ear vein B

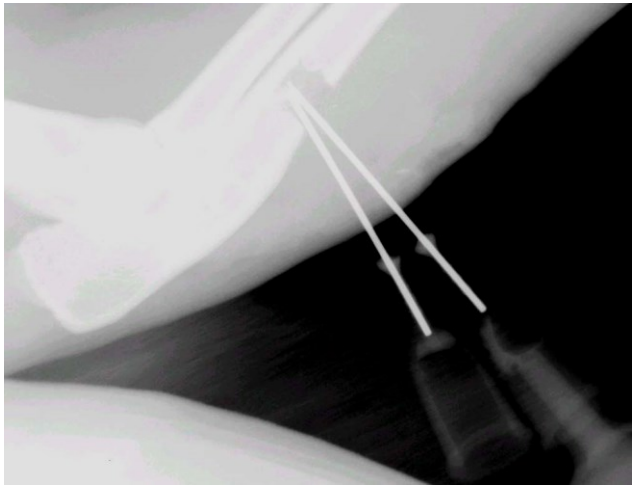
Fracture site A

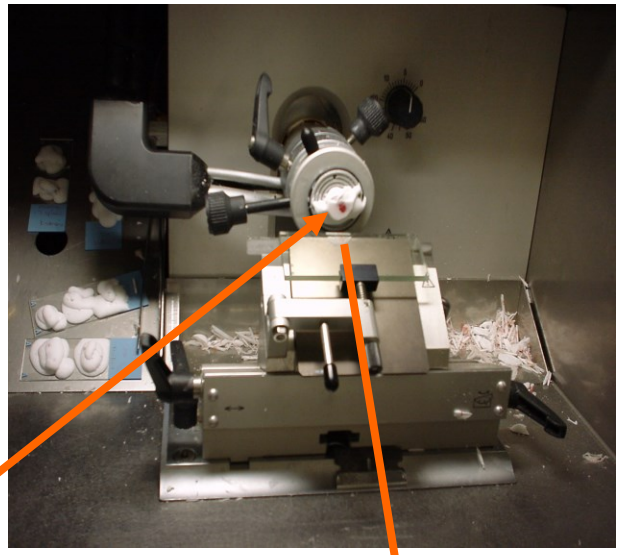
Remote BM site C

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

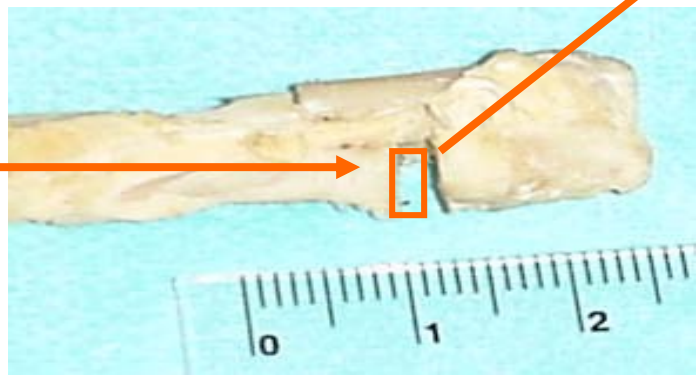
The tissues retrieved for frozen section – (5μm)

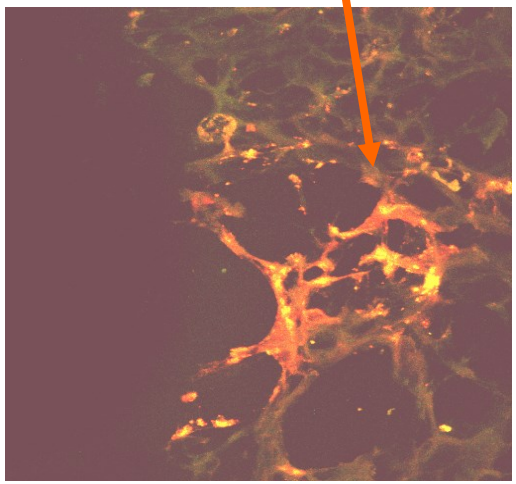
Animals were sacrificed at 3 and 12 weeks after cell implantation





Gap tissue



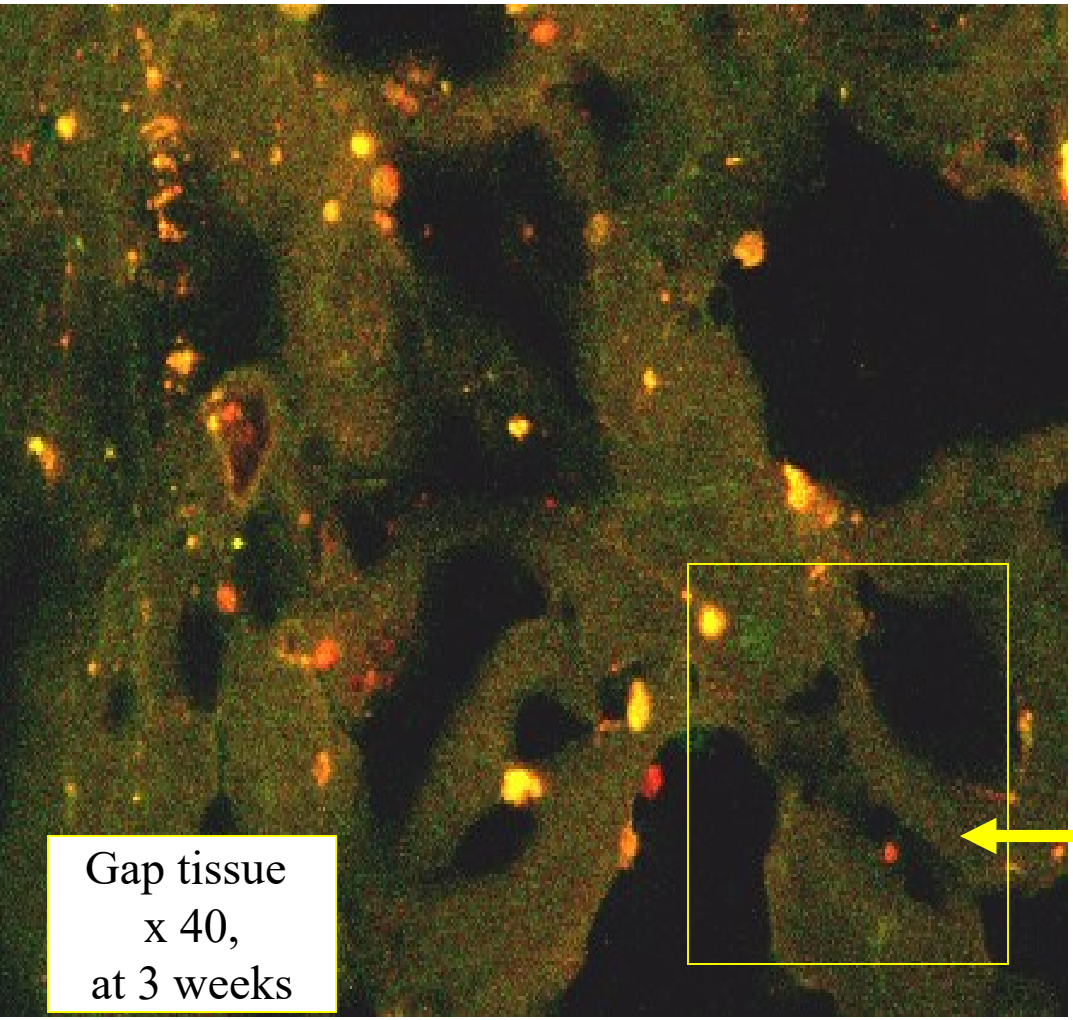


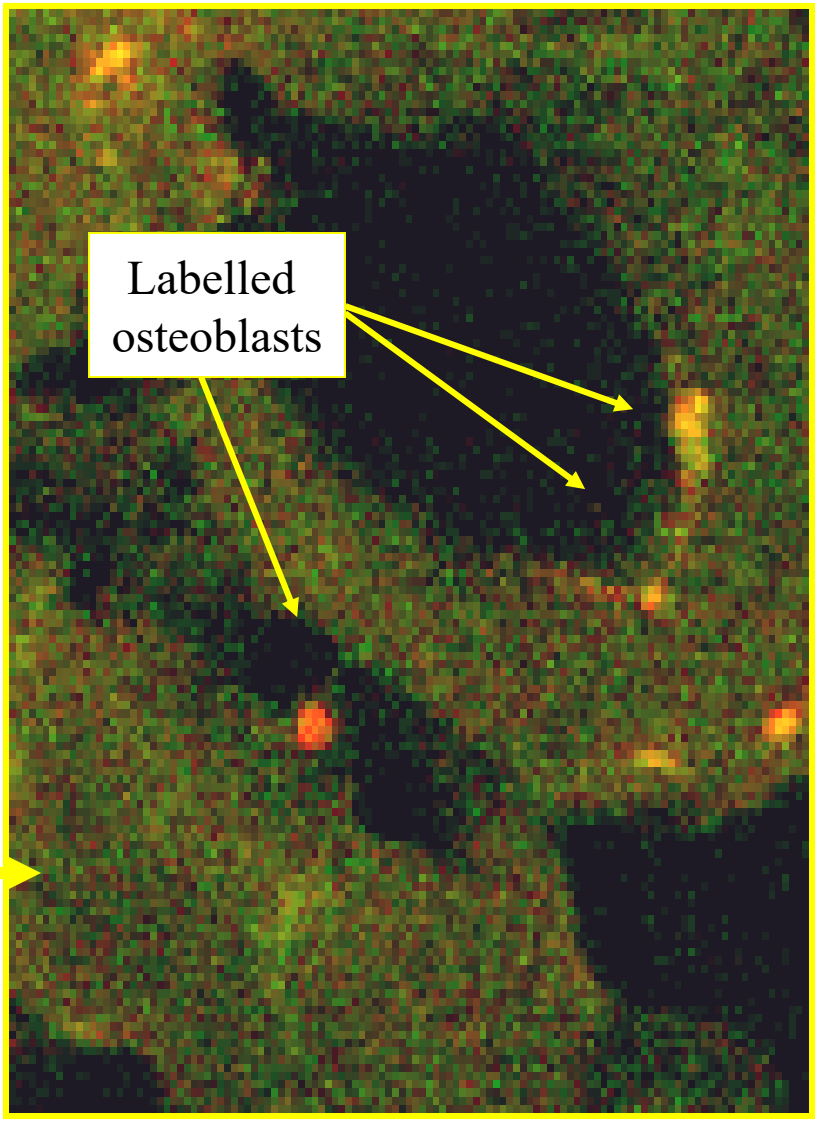
– Liver, lung, kidney, and spleen,

– Also cytospins of BM and blood

(representative samples only)

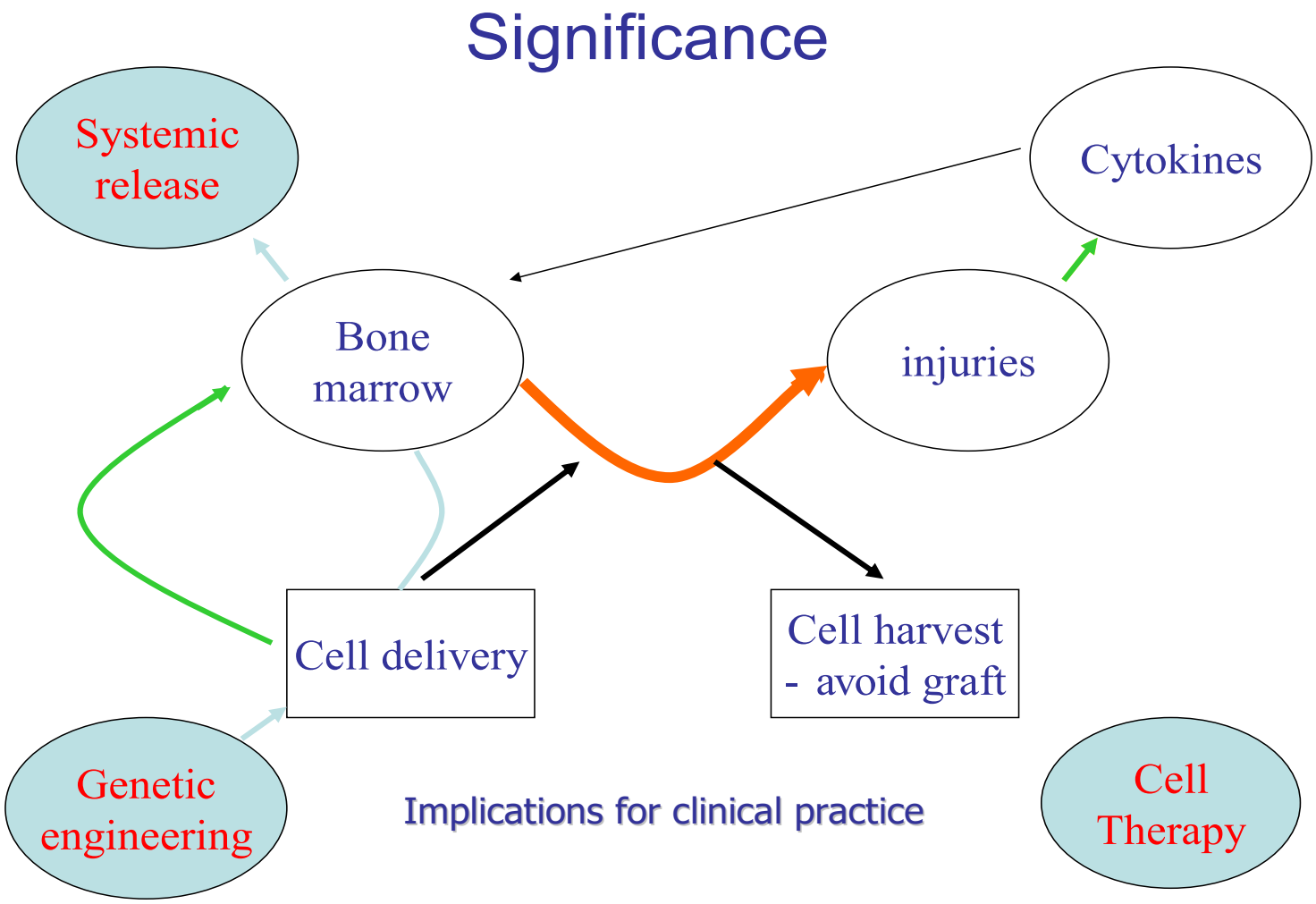
Labelled cells from remote marrow identified in fracture gap (Group C)





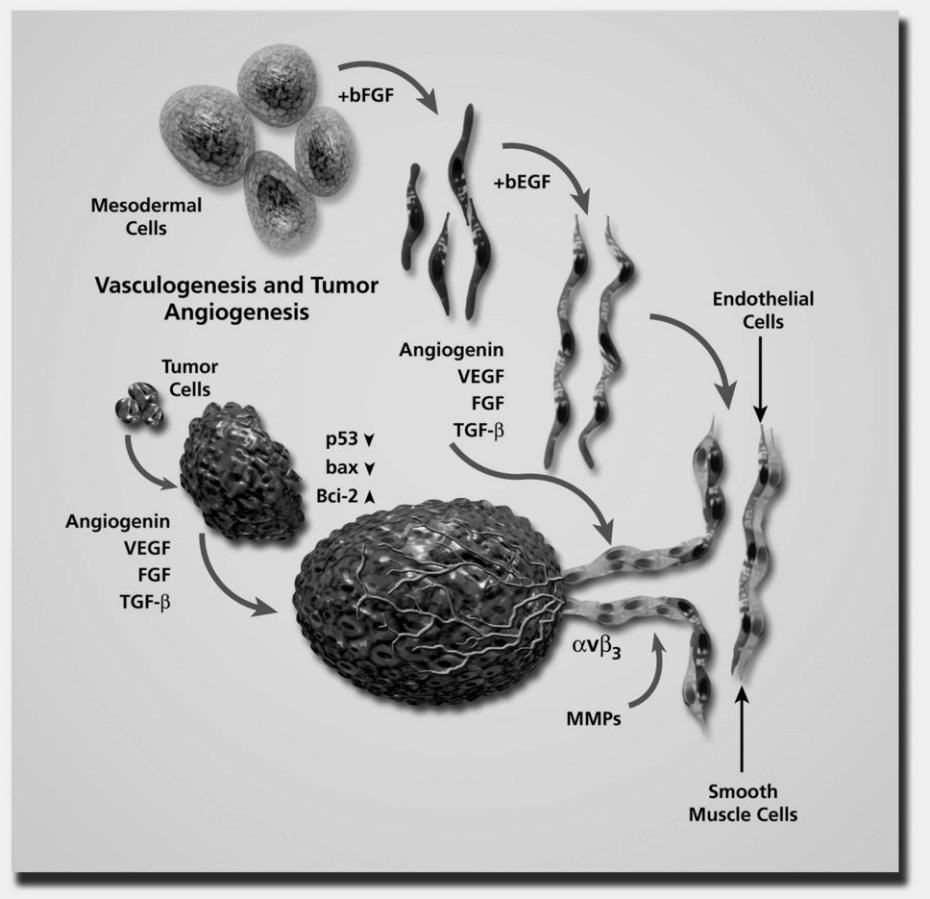
Shirley D, et al, JOR, 2005, 23 (5): 1013-21.

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

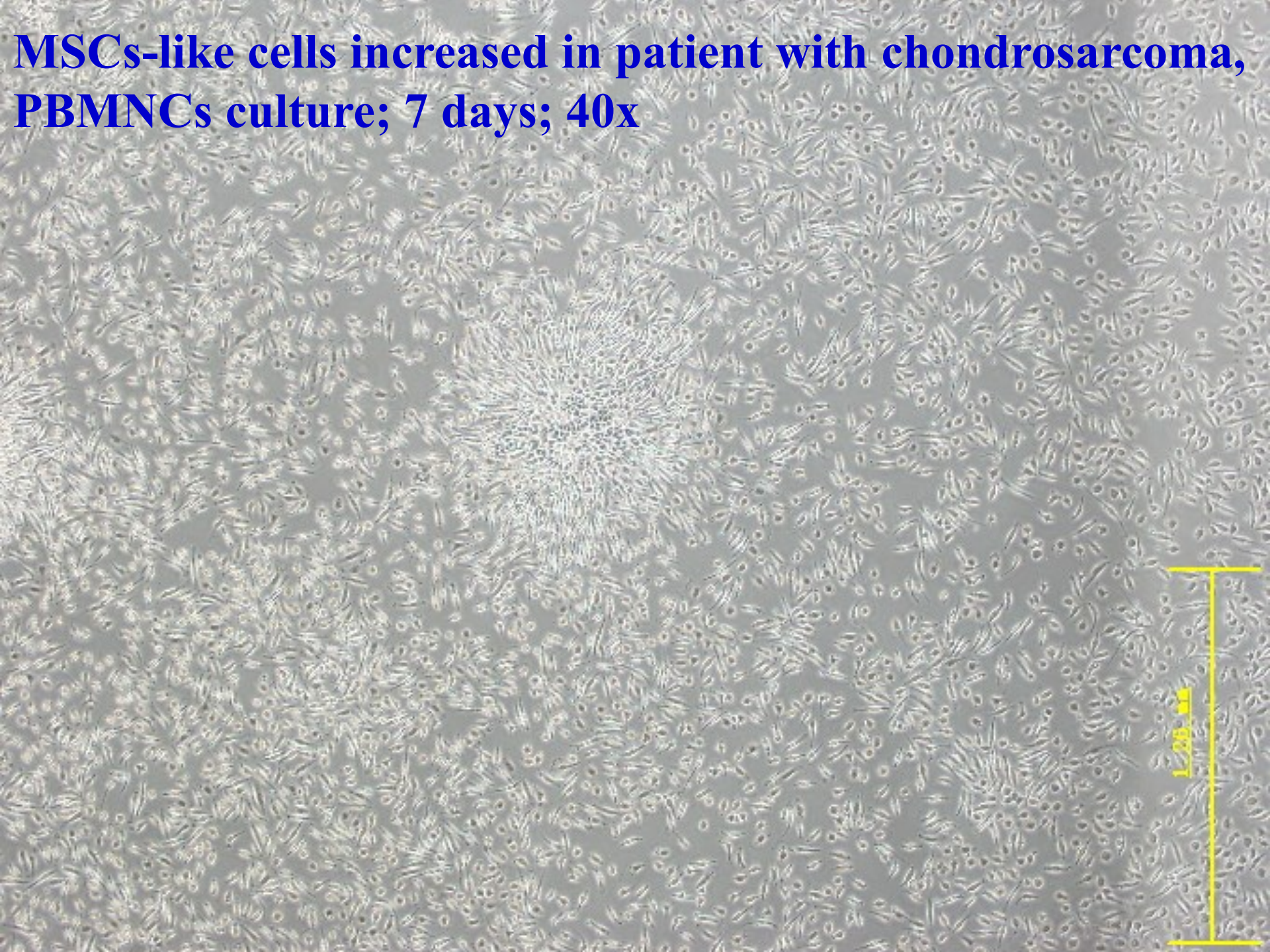


MSCs and Tumour Stroma

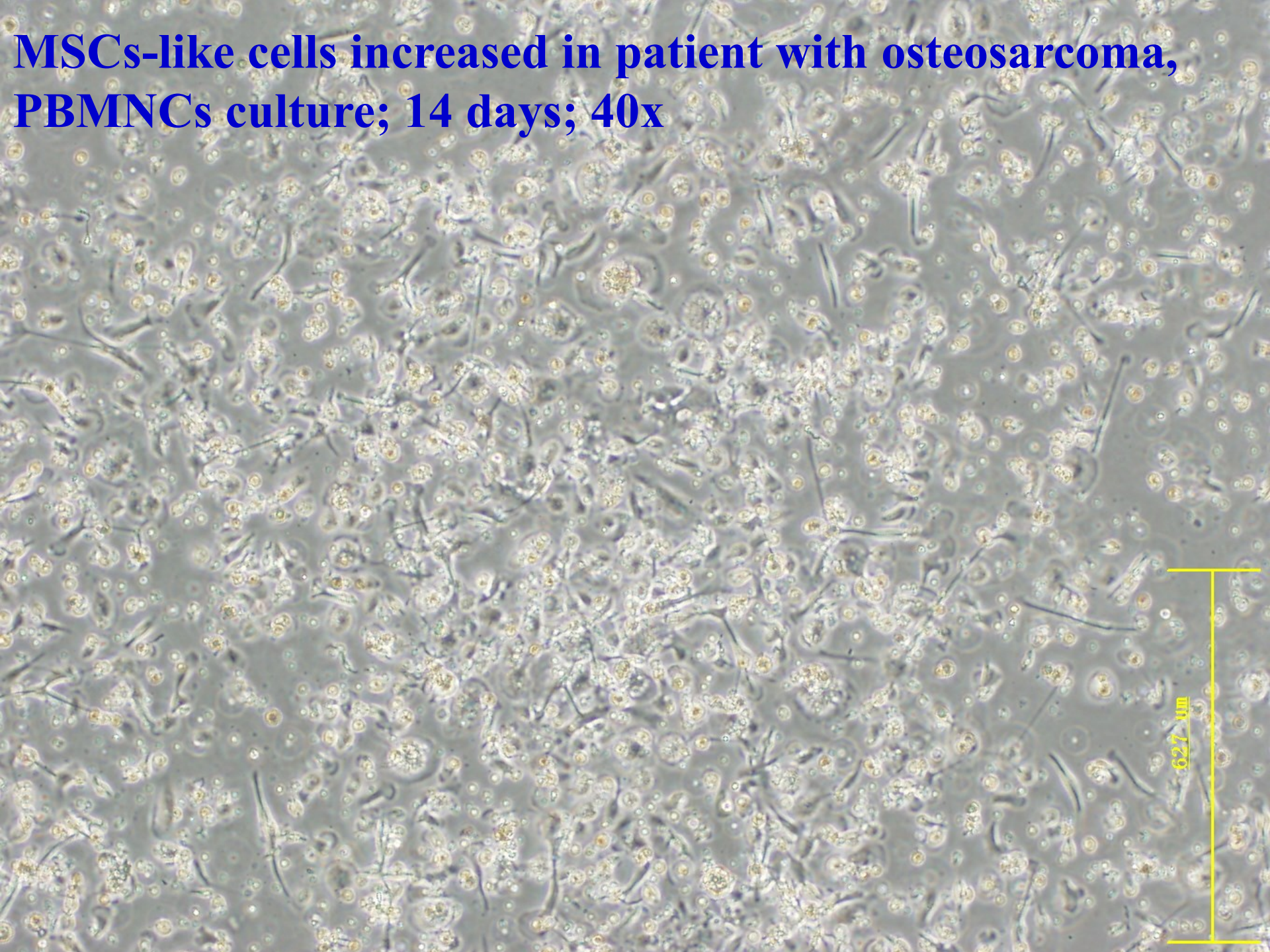
- **Where do the tumour stroma orginiate ?**
  - **Local**
  - **Circulating cells**
- **MSCs form tumour stroma.**
- **Tumor mcroenvironment select MSC engraftment.**



MSCs-like cells increased in patient with chondrosarcoma, PBMNCs culture; 7 days; 40x



MSCs-like cells increased in patient with osteosarcoma, PBMNCs culture; 14 days; 40x



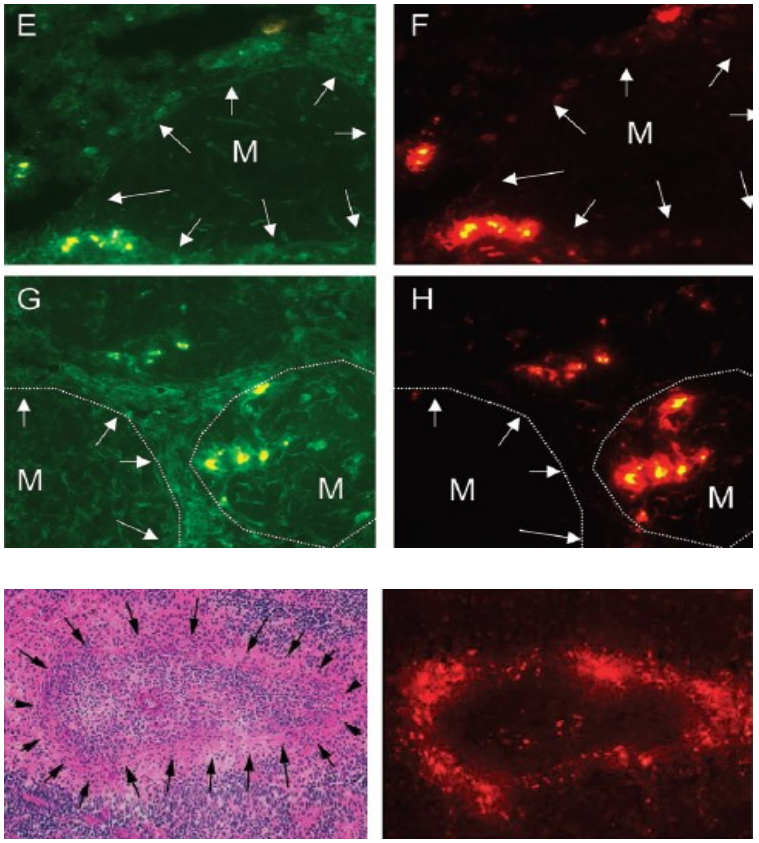
MSCs can home to the tumours

- Yes. MSCs can home to tumour sites in animal model

- Questions remain:

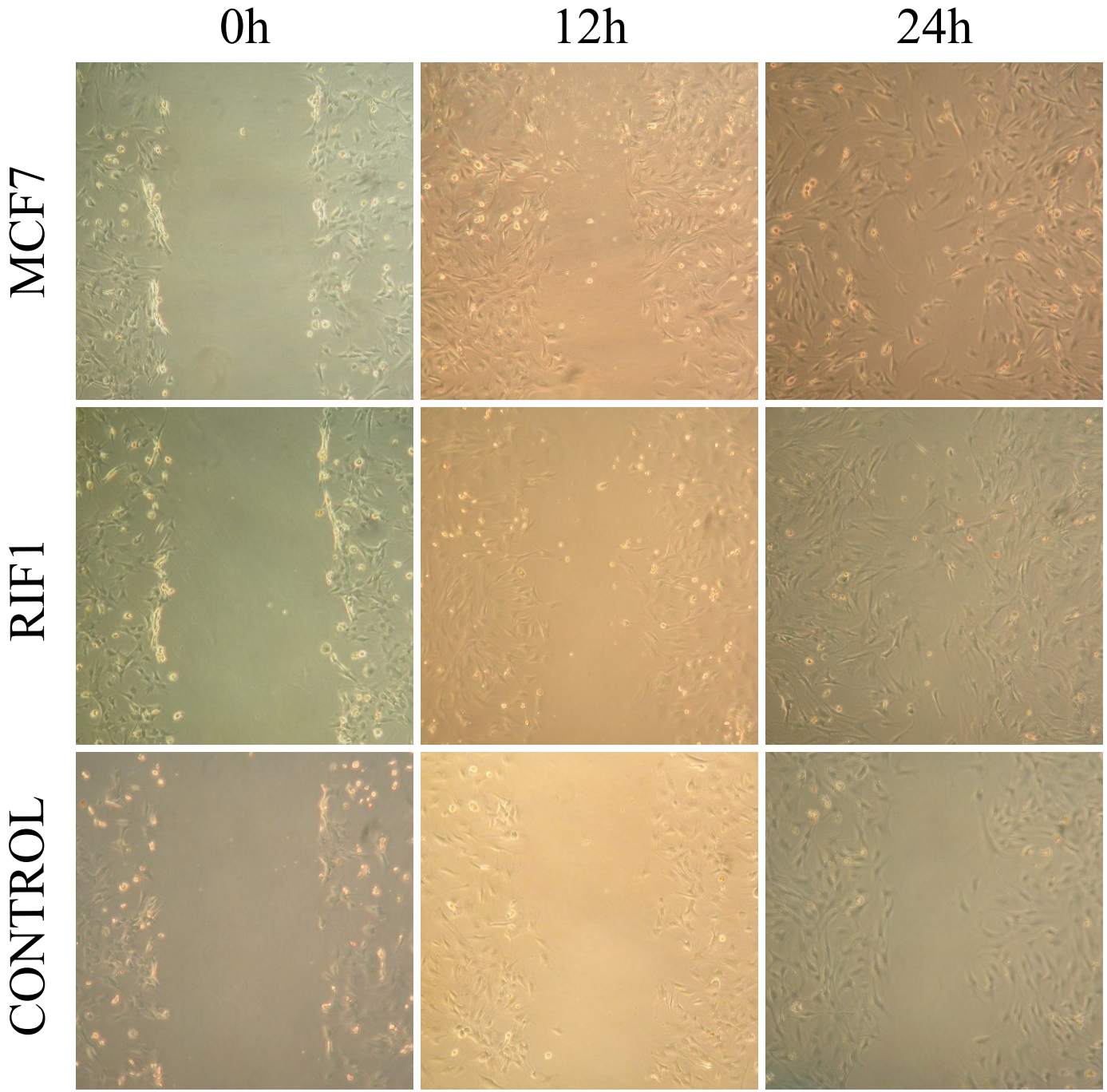
1. The fate of these MSCs

2. Need to be tested in different model systems



Journal of the National Cancer Institute, Vol. 97, No. 7, 2005

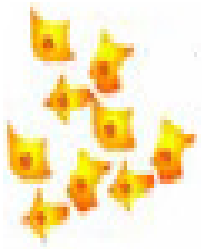
Wound healing assay of MSCs in condition medium tumor cell



Study MSCs Homing to Tumours

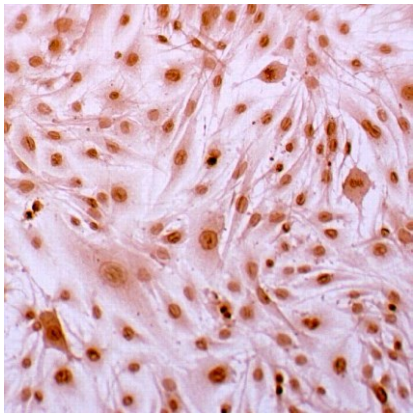
TUMOUR CELLS SUBCUTANEOUS IMPLANTATION

Luciferase labelled MSCs

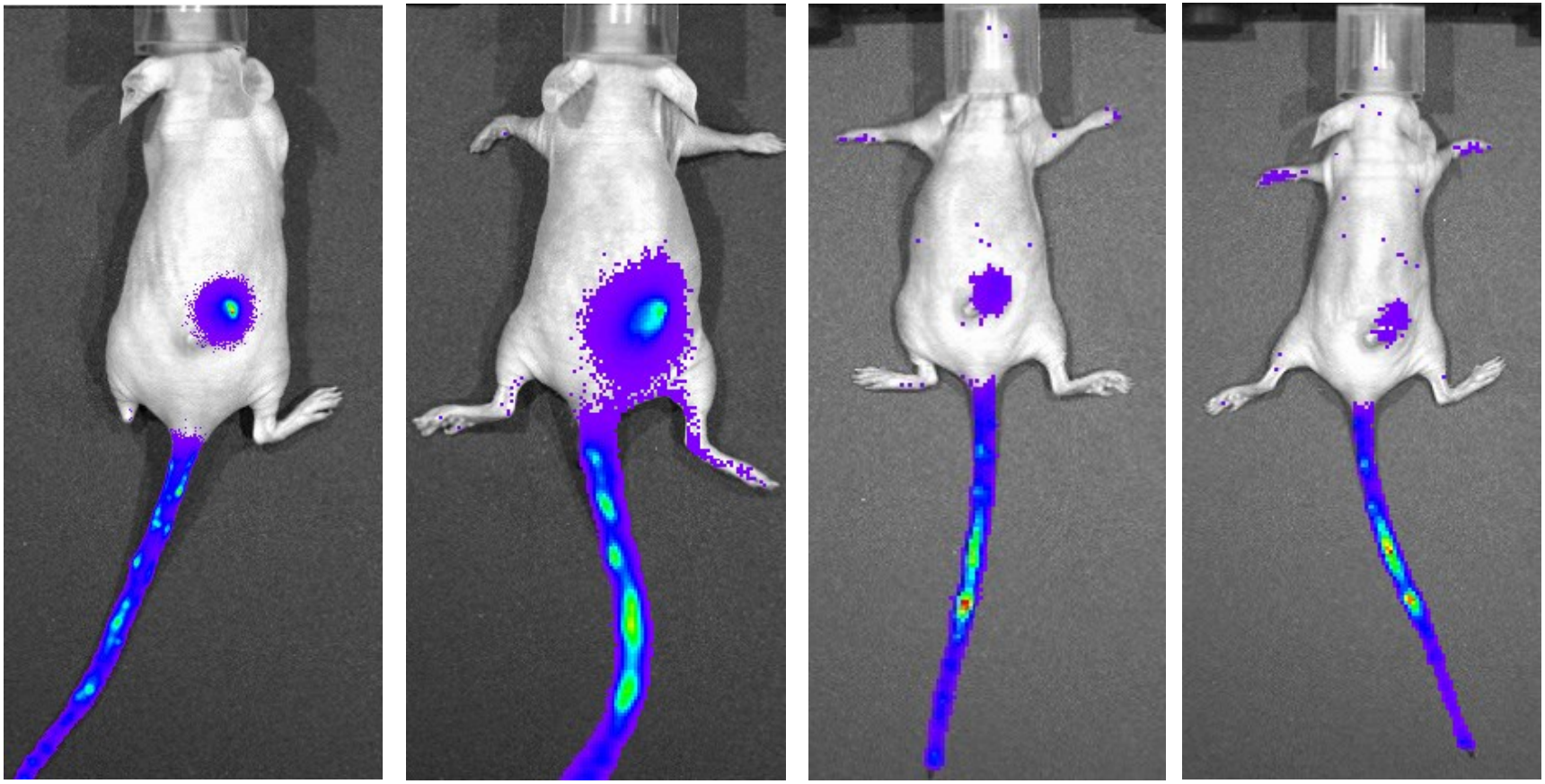


Tumour xenograft model

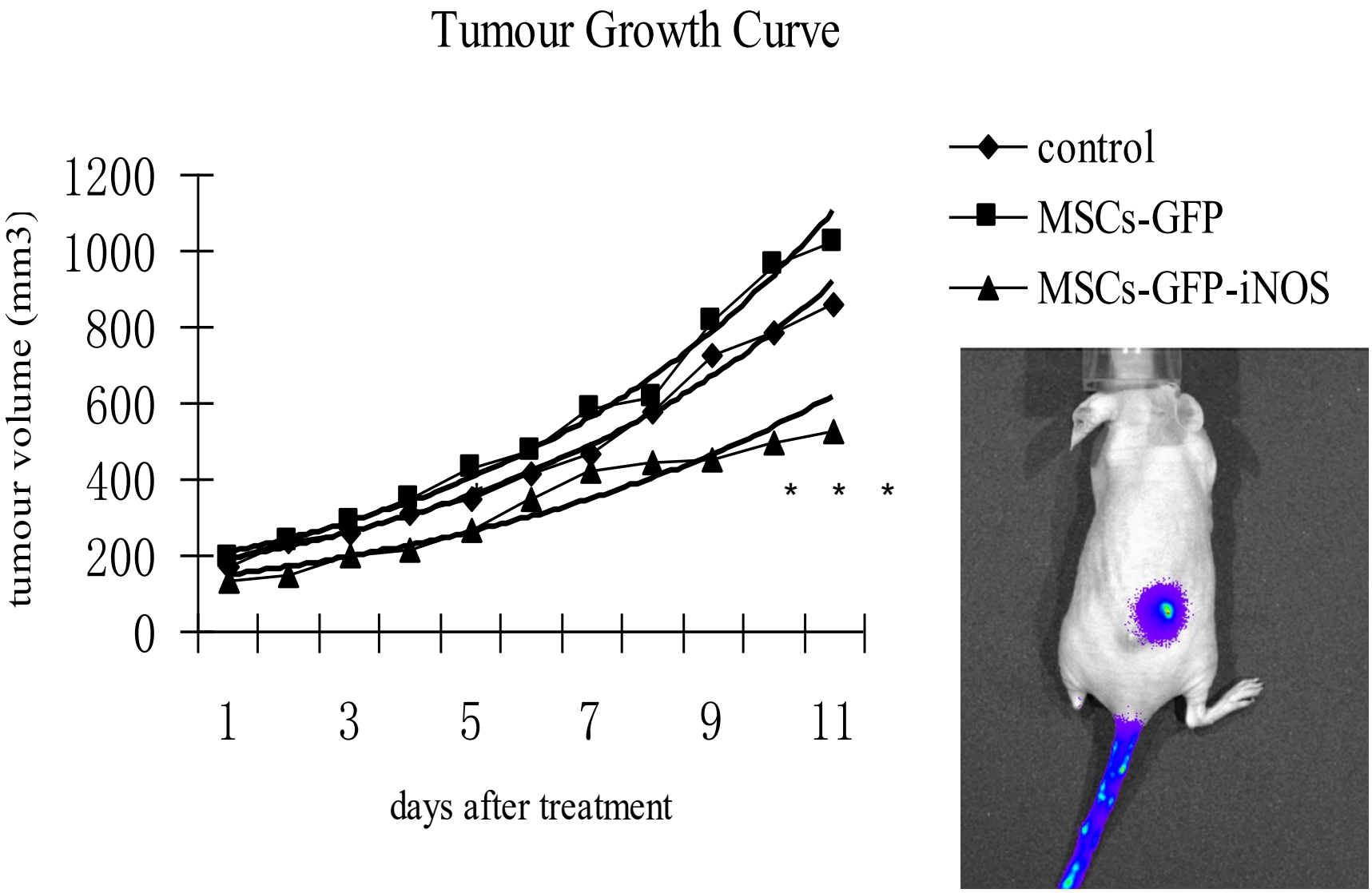
Luciferin injection
In-vivo imaging



MSCs distribution in tumor bearing mice



Day 3 Day 6 Day 9 Day 12

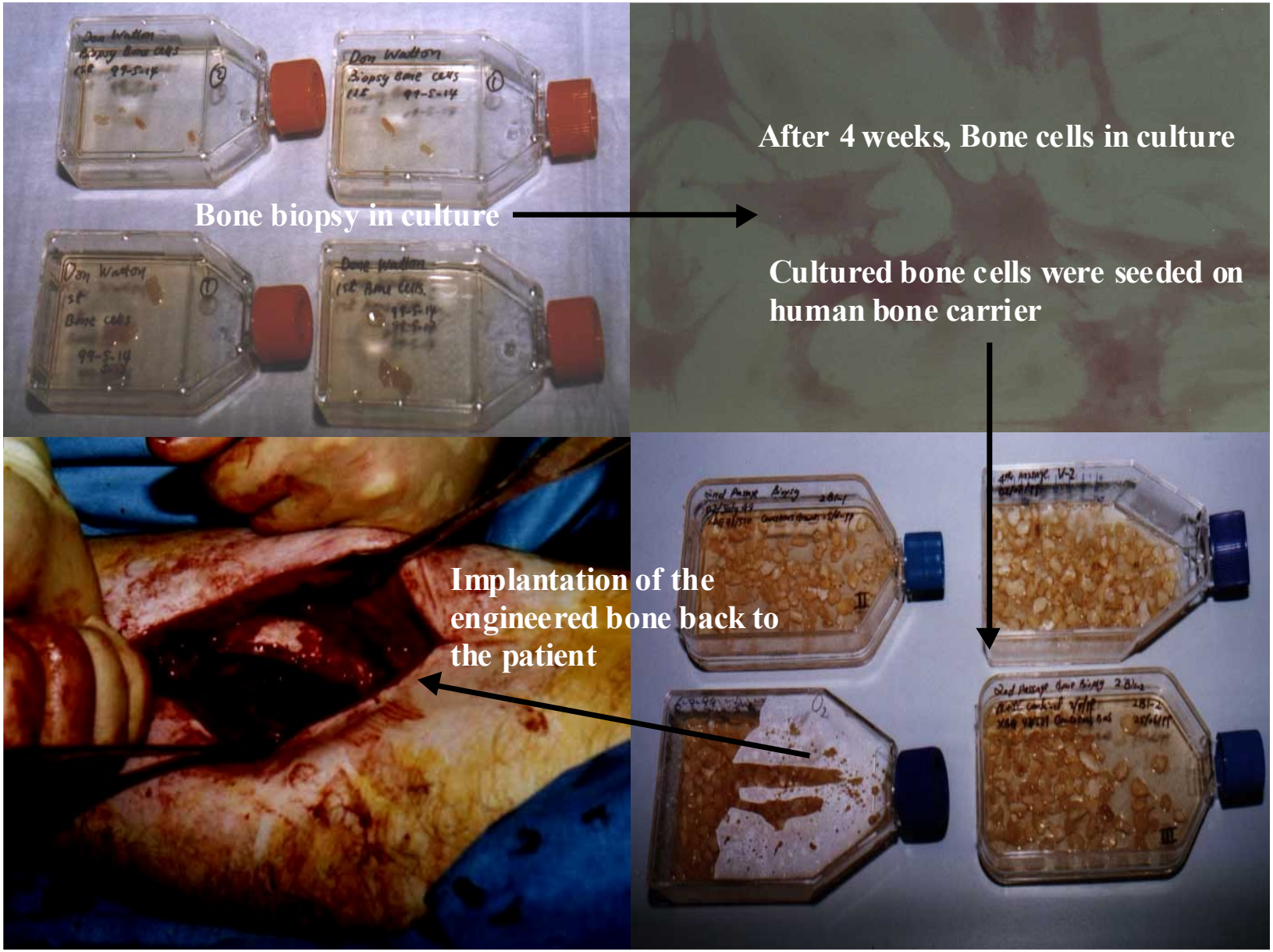


Systemic administration of MSCs-Lenti-iNOS significantly reduced fibrosarcoma growth in tumour bearing animals.

Outline of Discussion

1. Source of stem cells, allogenic MSCs and circulating MSCs.
2. MSCs culture and phenotyping techniques.
3. MSCs systemic recruitment and delivery.
4. Clinical Trials / safety and regulatory issues

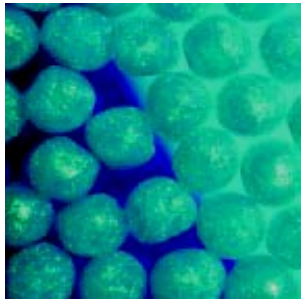
Clinical Trails of Using Autologous BM-MSCs for the treatment of bone defect



Cartilage Repair investigations:

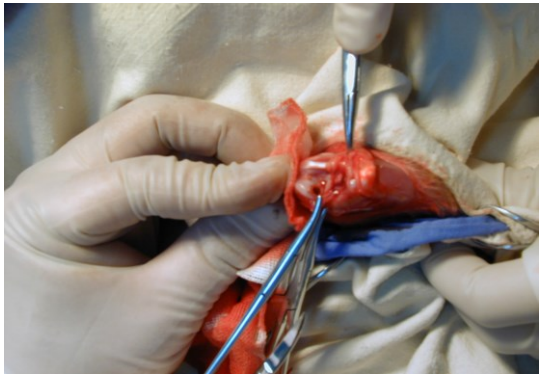
Repair of Cartilage by Bone Marrow MSCs over-expressing Sox-9 and Matrilin 3

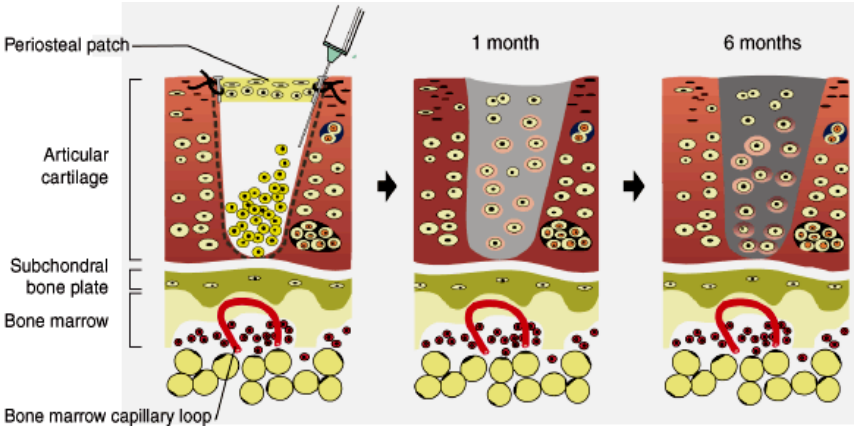
Select and expand bone marrow MSC clones with stably modulated levels of Sox9 and Matrilin 3 gene



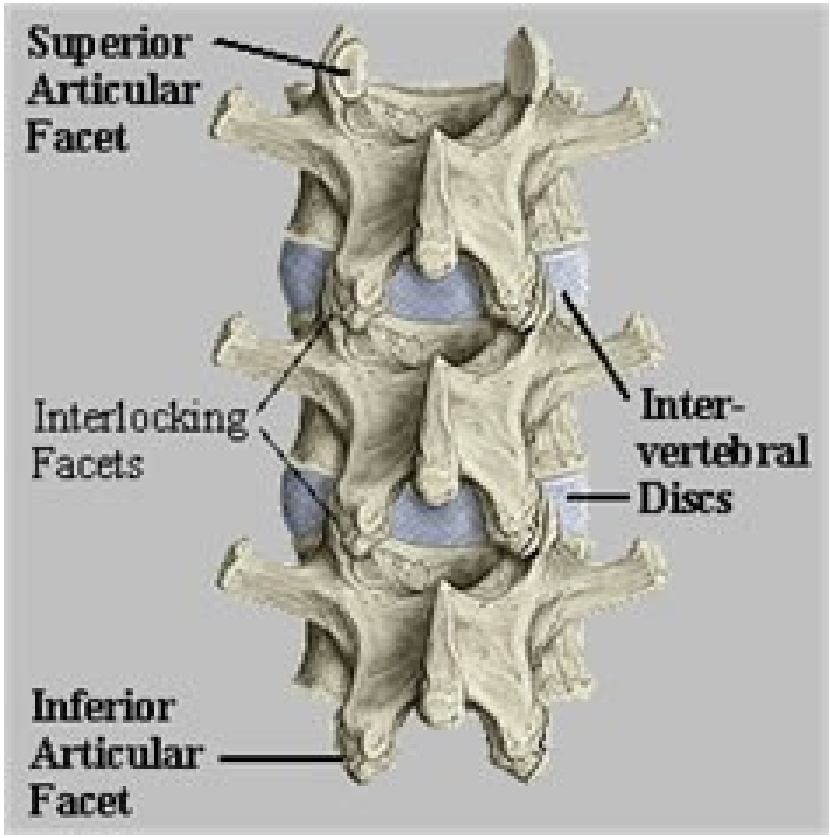
Implantation into rat models with cartilage defects

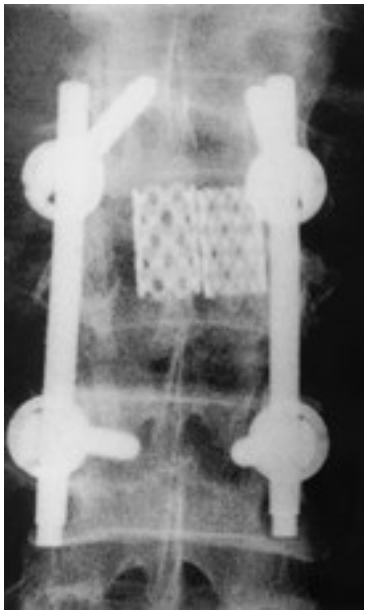
Cartilage repair: Immunohistochemical, histological and functional examinations

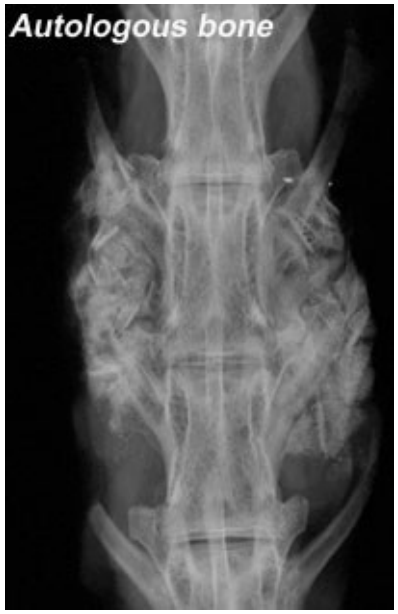




Spinal Fusion

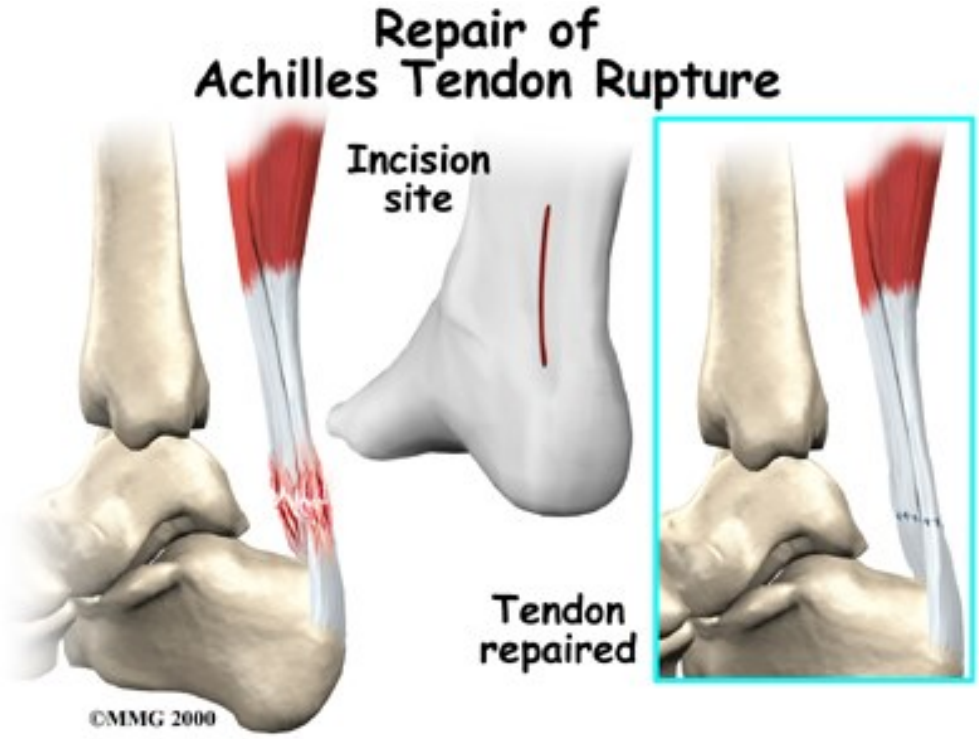


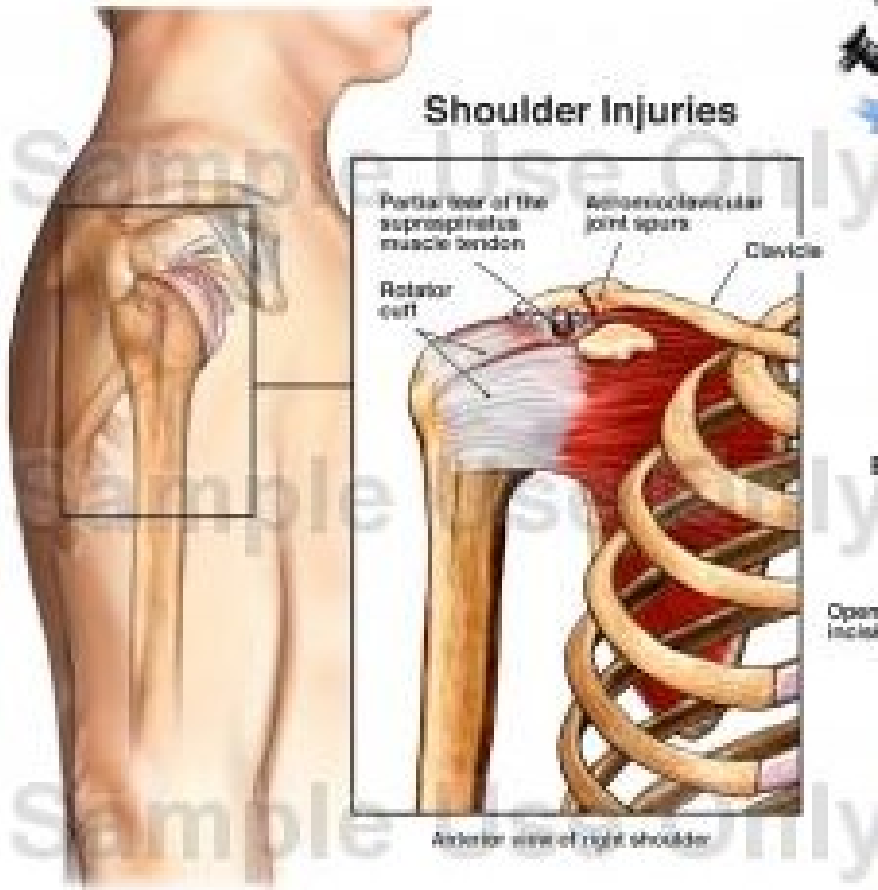




- Surgical stabilization
- Biomaterials
- Growth factors
- Cells

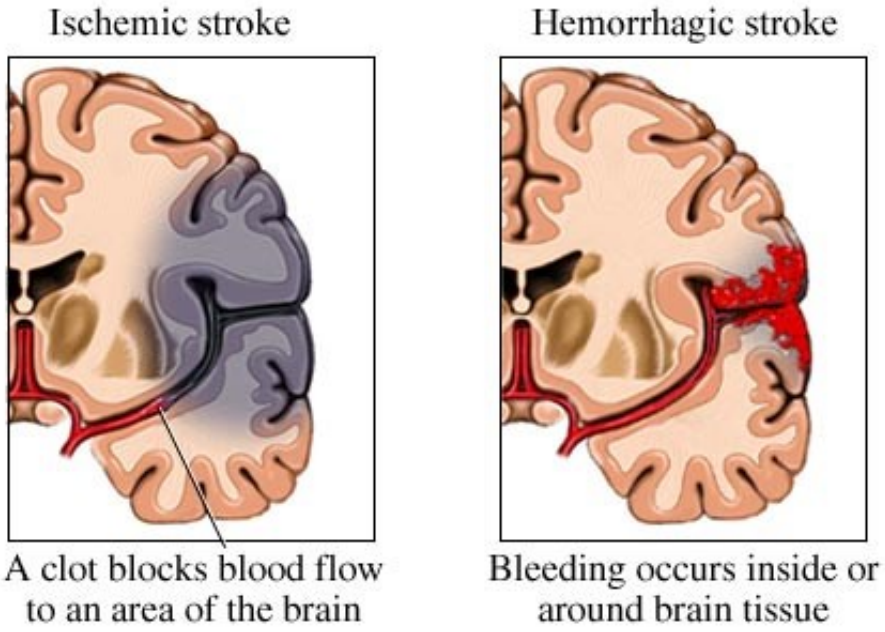
Tendon Repair

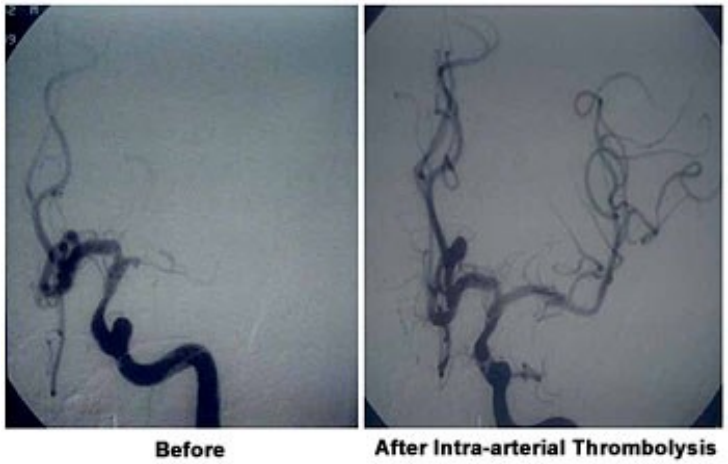




1. Tendon-derived MSCs vs BM-MSCs
2. Local delivery vs Systemic MSCs in tendon healing and tendnopathy models

Stroke Managements





Minimizing the damages

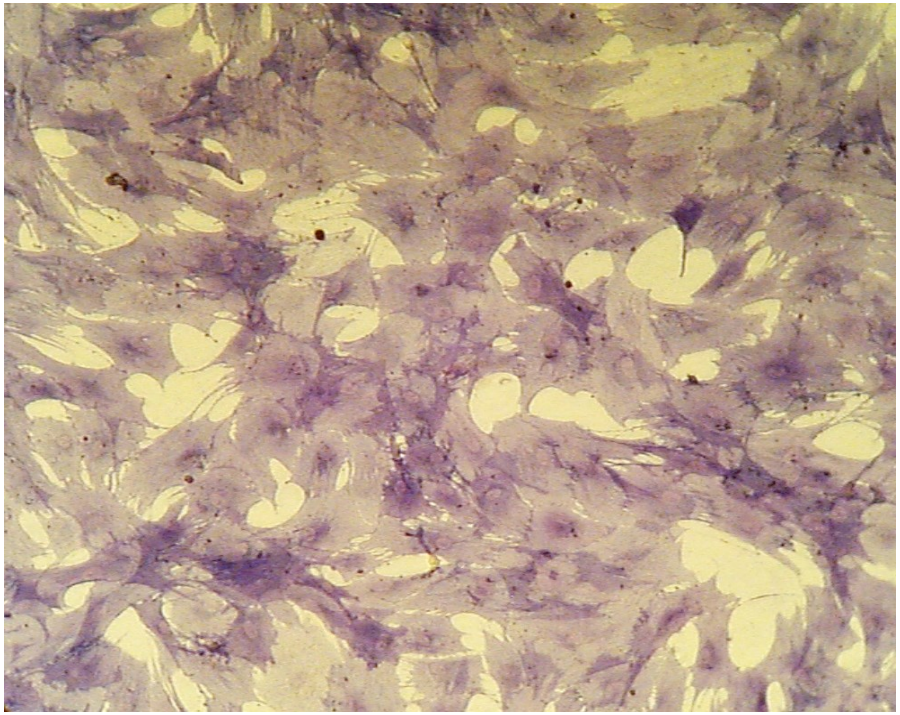
- **Stop bleeding**
- **Unblock the vessels**

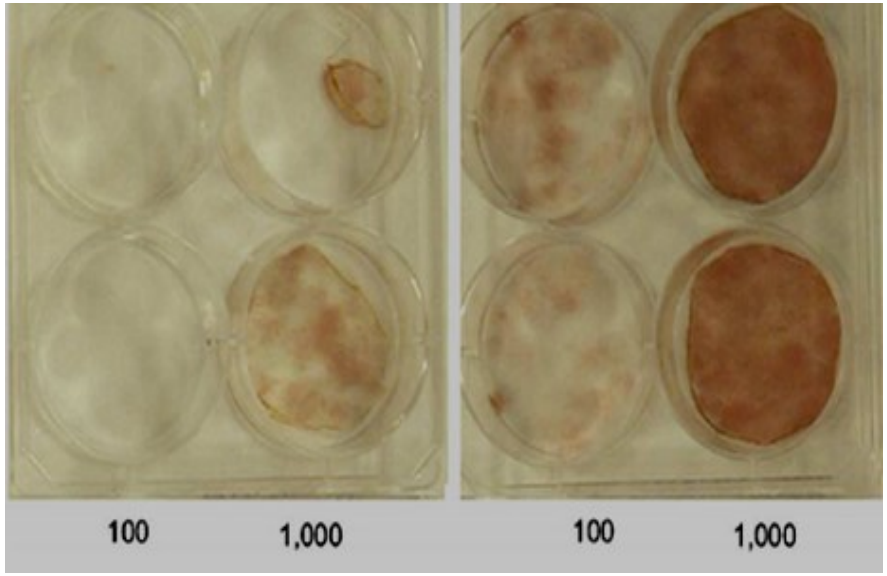
Maximizing the changes of functional recovery

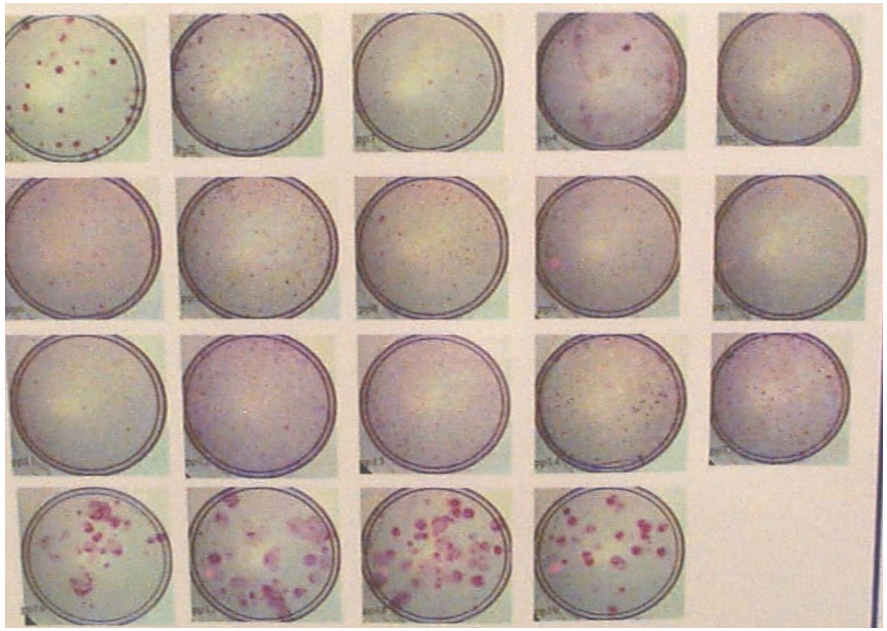
- **Control inflammation**
- **Restore blood supply**
- **Active physiotherapy**

Stem cells as measurement tools

- Mechanism of treatment
- Scanning tools- TCM, New drugs, methods







GMP Cell Culture Labs and Ethics

Investment in GMP Labs

Personnel

Local regulations

Ethical permissions

Patients Consents

Summary

- **Allogenic BM-MSCs, umbilical cord blood MSCs and tissue specific MSCs.**
- **Cell expansion techniques to allow rapid proliferation or controlled differentiation.**
- **Off shelf, ready to use cell products.**
- **Cell based gene therapy for enhanced and cost-effective tissue repair and regeneration.**
- **Aware the ethical and safety issues of cell therapy.**


Thank You !!
谢谢 !