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

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Bone Marrow Mesenchymal Stem Cells (MSCs)

- Multi-potent cells
- Easy to collect and expand
- Low immunogenicity
- Systemic recruitment
- Home to injury tissues
Concise Review: Multipotent Mesenchymal Stromal Cells in Blood

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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
Progress to date

153:1133-1139

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

human cells

Circulating osteoblast-lineage cells in humans.

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

PB-derived adherent, clonogenic, fibroblast-like cells

Osteogenesis in vivo; Adipogenesis in vitro

2:477-488

Osteogenesis in vitro
CD34-/CD45-/CD14-/CD3-
CD105+/type I collagen+

human

2000. Stem cells
18:252-260

CD34- /low
Osteocalcin+
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.

Blood → Lymphoprep → Before → After → Buffy coat → ICC

Centrifugation 400 g 20 min → Culture 2-4 weeks → RT-PCR
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

- Vimentin
- Osteocalcin
- Negative control
- Collagen 1
- BMPR 2
- CD 105
- BMP 2
### Summary of cell culture results

<table>
<thead>
<tr>
<th></th>
<th>&lt; 4 days Post-fracture</th>
<th>&gt; 14 days Post-fracture</th>
<th>Non-union patients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>cases</strong></td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><strong>cells</strong></td>
<td>few</td>
<td>Some</td>
<td>Many</td>
<td>None/few</td>
</tr>
</tbody>
</table>

![Cell images](image1.png) ![Cell images](image2.png) ![Cell images](image3.png)
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 found 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
Differentiation Potential of human blood-borne MSCs

In vitro angiogenesis

Matrigel 3D culture 24h × 100

Long term 2D culture 72h × 100

In vivo bone formation study

PBMCs seeded CaP block 3month × 50
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.


Where do circulating MSCs come from?

Systemic recruitment of osteoblastic cells in fracture healing

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Accepted 28 January 2005
MSCs homes to fracture sites through peripheral circulation

Bone marrow harvested

Rabbit bone marrow

MSCs culture

Re-implantation

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

Culture 3 weeks

Ulnar defect

BM harvest

48 hours post Fx

Control E

Ear vein B

Fracture site A

Remote BM site C

The tissues retrieved for frozen section – (5µm)

Animals were sacrificed at 3 and 12 weeks after cell implantation.

- 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)

Gap tissue x 40, at 3 weeks

• 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

- 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)

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS Heart Injection Group (Control)</td>
<td>0.5ml PBS/ Rat  was given at 4 days post-fx</td>
</tr>
<tr>
<td>MSCs Heart Injection Group</td>
<td>2x10^6 GFP-MSCs in 0.5ml PBS/ Rat was given at 4 days post-fracture</td>
</tr>
<tr>
<td>Fibroblast Heart Injection Group</td>
<td>2x10^6 GFP-Fibroblasts in 0.5ml PBS/ Rat was given at 4 days post-fracture</td>
</tr>
<tr>
<td>MSCs Fracture Site Injection Group</td>
<td>2x10^6 GFP-MSCs in 0.5ml PBS/ Rat was given at 4 days post-fracture</td>
</tr>
</tbody>
</table>
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

<table>
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<tr>
<th>PBS injection Group</th>
<th>MSCs Heart injection Group</th>
<th>Fibroblast heart injection Group</th>
<th>MSC local injection Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Fracture day" /></td>
<td><img src="image2" alt="Injection day" /></td>
<td><img src="image3" alt="1 week after" /></td>
<td><img src="image4" alt="2 week after" /></td>
</tr>
<tr>
<td><img src="image5" alt="3 week after" /></td>
<td><img src="image6" alt="4 week after" /></td>
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Fracture day
Injection day
1 week after
2 week after
3 week after
4 week after
RESULTS - COMPARING THE SIZE OF THE CALLUS
RESULTS: MICRO CT ANALYSIS

3D Reconstruction

Bone Volume / Total Volume (BV/TV)

- PBS Group: P=0.001
- MSC Group: P=0.001
- Fibroblasts Group: P=0.005
- MSC-Loc Group: P=0.002
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.
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.
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Thank You!