AMNIOTIC MESENCHYMAL STEM CELLS ENHANCE WOUND HEALING IN DIABETIC MICE THROUGH HIGH ANGIogenic AND ENGraTMENt CAPABILITIES

Plos One, 2012;7(7): e1105  Kim et al

AMM’s up regulate angiogenic factor IGF-1 EGF IL-8
Pomotes wound healing
increased re-epithelializtion by expressed keratinocyte-specific proteins and cytokeratin
Stem cells and wound healing

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Phases of wound healing in diabetes

Response to Diabetes

Inflammation  Regeneration  Fibrosis
## Mesenchymal Stem Cells Support All 3 Phases of Wound Healing

<table>
<thead>
<tr>
<th>Phase</th>
<th>Role of MSCs</th>
<th>References</th>
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</table>
| **Inflammatory Phase** | • Regulate inflammatory cells within wounds  
• Down regulate inflammation  
• Restore protease balance | Chen et al., 2008; Walter et al., 2010 |
| **Proliferative Phase** | • Production of key growth factors (VEGF, bFGF, PDGF) including those for angiogenesis  
• Attracts key cell types (keratinocytes, dermal fibroblasts, and host stem cells)  
**Promote cell to cell interaction and activity**  
**Release of growth factors important to wound healing** | McFarlin et al., 2006; Wu et al., 2007; Ishikane et al., 2008; Shin et al. 2012 |
| **Remodeling Phase** | • Promotes organized collagen deposition by dermal fibroblasts, reduces scaring  
• Regulates organization of ECM (MMPs and TIMPs)  
**Reduce fibrosis** | Tondreau et al., 2009; Walter et al., 2010 |
Microenvironment of chronic wounds
...opportunity for intervention

**HEALING WOUNDS**
- Apoptotic clearing (without necrosis)
- Mitotically competent cells
- Low Inflammatory cytokines
- Low Proteases, ROS
- growth factors

**CHRONIC WOUNDS**
- Senescent cells
- Inflammatory cytokines - Immunopathy
- Proteases- Degraded ECM* and growth factors
- Vascular, Infection, Pressure

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*ROS = Reactive oxygen species.*

†ECM = Extracellular matrix.
Mesenchymal Stem Cells

1. Stem cells are unspecialized
2. They have the ability to self-renew
3. They can change into mature cell types
### MSC occurrence in nucleated cells

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MSC proportion</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Marrow</td>
<td>~1 per 100,000-1,000,000 (1-30/cc)</td>
<td>Pittenger &amp; Martin, Circ Res, 2004</td>
</tr>
<tr>
<td>Trabecular Bone</td>
<td>~1 per 1,000-100,000</td>
<td>Sakaguchi et al., Arthritis &amp; Rheumatism, 2005</td>
</tr>
<tr>
<td>Adipose</td>
<td>~1 per 20,000</td>
<td>Sakaguchi et al., Arthritis &amp; Rheumatism, 2005</td>
</tr>
<tr>
<td>Synovium</td>
<td>~1 per 100</td>
<td>Sakaguchi et al., Arthritis &amp; Rheumatism, 2005</td>
</tr>
<tr>
<td>Periosteum</td>
<td>~1 per 100</td>
<td>Sakaguchi et al., Arthritis &amp; Rheumatism, 2005</td>
</tr>
<tr>
<td>Muscles</td>
<td>~1 per 100</td>
<td>Sakaguchi et al., Arthritis &amp; Rheumatism, 2005</td>
</tr>
<tr>
<td>Peripheral Blood</td>
<td>~1 per 1,000-10,000 (~1-4/cc)</td>
<td>Zvaifler et al., Arthritis Res., 2000</td>
</tr>
<tr>
<td>Skin</td>
<td>&lt;1:100 (in dermis)</td>
<td>Sellheyer &amp; Krahl, J Am Acad Dermatol., 2010</td>
</tr>
<tr>
<td>Cord Blood</td>
<td>~1 per 1,000 (100-1000/cc)</td>
<td>Paquet-Fifield et al., J Clin Invest., 2009</td>
</tr>
<tr>
<td>Amniotic Fluid</td>
<td><del>1 per 100 (</del> 0-5/cc)</td>
<td>Lu et al., Haematologica, 2006</td>
</tr>
<tr>
<td><strong>Amnion &amp; Chorion</strong></td>
<td><strong>1 per 1-4 (1-4x10^5/cm^2)</strong></td>
<td>Bieback &amp; Brinkmann, World J Stem Cells, 2010; Ilancheron et al, Placenta, 2009; Parolini et al., Stem Cells, 2008</td>
</tr>
</tbody>
</table>
Advanced release testing: cryopreserved cell viability

- Fluorescent microscopy of live/dead staining depicts microstructure
- Cell viability tested and assured after freezing

**Passing Final Product**

**Failing Product**

Green = viable cells, calcein-AM

Red = non-viable cells, ethidium homodimer-1
Composition of Amniotic MSC

Acellular Skin Substitutes
- Collagen Containing Matrices
  - Collagen Rich Matrix
- Devitalized Allografts
  - Growth Factors
  - Collagen Rich Matrix

Cellular Autologous and Allogeneic Skin Substitutes
- Epithelial Cells and/or Fibroblasts
  - Epithelial Cells
  - Growth Factors
  - Collagen Rich Matrix

MSCs
- Epithelial Cells
- Fibroblasts
- Growth Factors
- Collagen Rich Matrix
Amniotic MSCs Provides Sustained Release of Growth Factors

PDGF-BB

HGF

Arnold SAWC September 2013
MSCs Are Responsive to the Microenvironment

- VEGF is important in promotion of angiogenesis
- In a hypoxic environment, MSCs up-regulate vascular endothelial growth factor (VEGF)

Response to hypoxia

Arnold et al., The Symposium on Advanced Wound Care Meeting, September 2013. Las Vegas, Nevada
Levels of Evidence Amniotic MSC

- Randomized clinical trial
- Prospective, blinded series
- Prospective, non-blinded series
- Retrospective series
- Case report
- Authority
- Anecdotal
- Animal Studies
Human Chronic non-healing wounds (n = 3) Autologous bone marrow aspirate and cultured MSCs Subcutaneous injection of bone marrow aspirate and 1–3 topical applications of MSCs Complete closure of wounds. Increased inflammatory response and angiogenesis. Badiavas and Falanga, 2005

Human Chronic non-healing wounds (n = 24) Autologous P0 MSCs Intramuscular and subcutaneous injection of > 1 × 10⁶ cells/cm² ulcer area and topical application Decreased wound size. Increased pain-free walking distance. Dash et al., 2007

Human Acute (n = 4) and Chronic (n = 6) non-healing wounds Autologous P2-10 MSCs 1–3 topical applications by fibrin spray Complete healing of acute wounds. Reduction or complete closure of chronic wounds. Dose dependent effect. Falanga et al., 2007

Human Chronic non-healing wounds (n = 20) Autologous P0 MSCs Topical application of MSC seeded collagen sponge Complete closure of 13 wounds. Partial closure of five wounds. Yoshikawa et al., 2008
A retrospective analysis of a human cellular repair matrix for the treatment of chronic wounds

Regulski M, Ostomy Wound Manage 2013 Dec;59(12):38-43

Median time to closure is 5.6 weeks with median 3 applications

> 70% probability of closure at 12 weeks
Tissue Source of Mesenchymal Stem Cells

MSC tissue

MSC Solution