Wound Healing & Limb Salvage

Dr. Harold Brem
The Wound Healing Program
Columbia University Medical Center
November 8, 2006
NJ Center for Biomaterials Institute: Wound and Limb Salvage Program

- Identifying the Problem of Limb Loss
- Healing Traumatic Wounds
- Utilizing Tissue Engineering to Prevent Limb Loss
- WEMR: Telemedicine to Accelerate Closure
- Data Bank for Traumatic Wounds
- Future
  New Modalities:
  - Sustained Release
  - Angiogenesis
  - Human Cell Bank
  - Molecular Basis of Healing
  - Limb Regeneration
February 12, 2006

The Wounded; Healing, With New Limbs and Fragile Dreams

By JULIET MACUR; Correction Appended

It was a victory for Lance Cpl. Matthew Schilling to walk into the upper gallery of the House of Representatives on Jan. 31 for the State of the Union address. He wore his dress blues and a prosthetic leg. Five months earlier, he had been carried on a stretcher, wounded and bleeding, into a hospital in Iraq after a roadside bomb exploded 10 feet from him…. The blast tore through his right foot and calf……

Corporal Schilling’s Marine Corps unit and a victim of the same blast, Lance Cpl. Mark Beyers, wheeled up to him at the Walter Reed physical therapy clinic. Corporal Beyers’s right arm and leg were amputated in Iraq. Since Aug. 26, when they were wounded, the two marines each have endured some 20 operations in three countries. Charting their care over the ensuing months, beginning just hours after the blast, has revealed a journey of medical setbacks and emotional turmoil. Among the more than …

About an hour later, Corporals Schilling and Beyers were in surgery at the nearby Al Asad Military Base. It was the first of 13 operations they would endure in eight days, during stays at five hospitals in three countries. A doctor amputated Corporal Schilling’s right leg below the knee. Corporal Beyers, with severe lung injuries, was in worse shape. Doctors amputated his right lower leg and his entire right arm, including the shoulder; shrapnel had destroyed his shoulder joint and just missed slicing his carotid artery, doctors said. For the next week he would be in an induced coma … After 19 operations and 63 days in hospitals, Corporal Beyers went home in October for a visit.
...16,653 Americans wounded in Iraq are 387 amputees, including 62 who, like Corporal Beyers, have lost more than one limb, said Lt. Col. Paul Pasquina, chief of physical medicine and rehabilitation at Walter Reed. The amputations, traumatic though they are, are often accompanied by painful complications. "It's not as easy as putting on even the most high-tech prosthetic and just walking off," Colonel Pasquina said....
### What is the amputation rate in battle injuries?

<table>
<thead>
<tr>
<th>War</th>
<th>Raw percentage</th>
<th># amputees</th>
<th>% multi-limb amputation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Civil War</td>
<td>12</td>
<td>~50,000</td>
<td>Unknown</td>
</tr>
<tr>
<td>WW I</td>
<td>1.7</td>
<td>2610</td>
<td>27</td>
</tr>
<tr>
<td>WW II</td>
<td>1.2</td>
<td>14,912</td>
<td>7</td>
</tr>
<tr>
<td>Korean War</td>
<td>1.4</td>
<td>1477</td>
<td>8</td>
</tr>
<tr>
<td>Vietnam</td>
<td>3.4</td>
<td>5283</td>
<td>20</td>
</tr>
<tr>
<td>Iraq</td>
<td>2.3</td>
<td>381</td>
<td>16</td>
</tr>
</tbody>
</table>

441 limbs lost (337 lower extremity) in war on terrorism alone!

WHAT CAN BE DONE?

Wound Healing Program at Columbia University

- Dedicated Inpatient Unit: New York Presbyterian Hospital
- Outpatient Wound Center: Case Management
- Clinical Research, Mailman School of Public Health: Outcomes
- Research Laboratory: College of Physicians & Surgeons
Healing traumatic wounds: Could this have been avoided?
Can a leg ulcer heal with human cell therapy?

YES!

Ulcer healed at 7 weeks.

**Leg Ulcer Protocol**

Venous ulcers are commonly on the ankle, but include any area below the knee that has + reflux (e.g. the foot)

---

**Initial Visit**

- **Laboratory Tests**
  - CBC with Manual Diff
  - Platelets
  - Hemoglobin A1C
  - ESR
  - PT/PTT
  - Albumin
  - Prealbumin
  - Na
  - K
  - Cl
  - CO₂
  - BUN
  - Creatinine
  - Glucose

- **Weekly Digital Photography, Objective Measurement**

- **Topical Therapy**
  - (1) Evaluate
  - (2) Treat
  - Debridement

- **Physical Exam**
  - Cellulitis
  - Fungal Nails
  - Drainage*
  - >2 months duration

- **Venous Reflux**
  - (1) can pick up clot in deep vein
  - (2) demonstrates blood flowing from deep to superficial vein
  - (3) shows perforating veins

- **Arterial Brachial Index**
  - If ABI >1.2
  - Pulse Volume Recording
  - Vascular Surgery
    - (1) Vascular Consult
    - (2) MRA Angiogram
    - (3) Angioplasty
    - (4) Stent Bypass

- **Deep Culture**
  - Antibiotics
    - No cellulitis
    - Minimal/decreased drainage*
    - 4-Layer Compression

- **Pathology**
  - Evaluate for:
    - (1) Tumor
    - (2) Infection
    - (3) Necrosis
    - (4) Scar

---

*Drainage is often a sign of infection, in which case compression is not advisable.*

What is Cell Therapy?

cell therapy in Petri Dish
Lifted with Forceps
Saline Irrigation
Fibroblast
Keratinocyte Layer
What Quality Control is There to Assure the Cells Are Living?

Hematoxylin and eosin (H&E) stained is done as part of quality assurance before shipping for use in a patient.

Use of Tissue Engineered Skin Already Shows Clinical Success in Promoting Wound Healing

Progression of Healing in a Venous Ulcer After Extensive Debridement and Apligraf Treatment

What is Clinical Presentation of the non-healing Wound?

Initial presentation of diabetic foot ulcer

Appears healthy, significant undermining

Callous is indication for debridement

Rapid healing with early treatment after Cell therapy

Can toe ulcers be a source of amputation?

Non-neuropathic “DFU”

Immediate treatment with cell therapy

Early treatment prevents amputation

What is the mechanism of cell therapy? Clue comes from wounds with large deficits.

Four Days After cell therapy

Healed at 49 Days

Mechanism of Cell Therapy: Keratinocytes and Fibroblasts release at least 17 growth factors

Importance of protocol for Foot Ulcer x 17 years?

- Looks good but Physiologically impaired
- Sewing cell therapy to New Wound Edge
- Sutured 1-mm inner Wound Edge so Keratinocytes are stimulated
- Application of Redundant cell therapy

New Epithelial Edge

Healed at 20 Weeks
Is Amputation Necessary?

Do Co-morbidities Stop a Wound From Healing?

# Healing rate of cell therapy in complicated leg ulcers?

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Center A (n=32)</th>
<th>Center B (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound Size (cm²)</td>
<td>Mean 39 ± 68</td>
<td>65 ± 21</td>
</tr>
<tr>
<td></td>
<td>Median 12</td>
<td>12</td>
</tr>
<tr>
<td>Wound Duration (years)</td>
<td>Mean 8.2 ± 9.4</td>
<td>7.6 ± 7.1</td>
</tr>
<tr>
<td>Median (years)</td>
<td>Median 5</td>
<td>4.2</td>
</tr>
<tr>
<td>Number of Applicatoins</td>
<td>2 ± 1</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Median 2</td>
<td>2</td>
</tr>
<tr>
<td>Time to Healing (days)</td>
<td>Median 61 ± 12</td>
<td>55 ± 14</td>
</tr>
<tr>
<td></td>
<td>Median 55</td>
<td>31</td>
</tr>
</tbody>
</table>

H. Brem, MD, Balledux J, Falanga V, et al

*Dermatologic Surgery* 2001, p. 915-921
Can Telemedicine Accelerate Wound Closure: WEMR
What is the importance of the Wound Electronic Medical Record (WEMR) for accurate data?
How do you interpret data? Percentage of Healing
Patients
703 Wounds, 431 Patient Visits
Q/A Non Healing Wounds

<table>
<thead>
<tr>
<th>Visit Date</th>
<th>Number of Wounds</th>
<th>PU I</th>
<th>PU II</th>
<th>PU III</th>
<th>PU IV</th>
<th>DF</th>
<th>VSU</th>
<th>Art</th>
<th>Surg</th>
<th>Other</th>
<th>Non-Healing Wound</th>
<th>Percentage of HEALING</th>
</tr>
</thead>
<tbody>
<tr>
<td>07_01_05</td>
<td>155</td>
<td>31</td>
<td>1</td>
<td>10</td>
<td>7</td>
<td>13</td>
<td>21</td>
<td>74</td>
<td>25</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>07_08_05</td>
<td>139</td>
<td>24</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>19</td>
<td>7</td>
<td>83</td>
<td>18</td>
<td>3</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>07_15_05</td>
<td>134</td>
<td>23</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>17</td>
<td>12</td>
<td>69</td>
<td>16</td>
<td>3</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>07_22_05</td>
<td>128</td>
<td>12</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>9</td>
<td>5</td>
<td>80</td>
<td>20</td>
<td>4</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>07_29_05</td>
<td>147</td>
<td>28</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>21</td>
<td>12</td>
<td>76</td>
<td>21</td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>JULY TOTALS</td>
<td>703</td>
<td>118</td>
<td>3</td>
<td>22</td>
<td>14</td>
<td>79</td>
<td>57</td>
<td>382</td>
<td>100</td>
<td>16</td>
<td>30</td>
<td>43</td>
</tr>
</tbody>
</table>
What are the clinical signs of a wound infection?

8/6/06

8/9/06
How much does a non healing wound cost? The James Peters VA experience

Can large ulcers heal?

Demographics:
145 Consecutive Hospitalized Patients

- Institutional Review Board consent to measure rate of healing and take photographs
- Age: 62.52 years
- Albumin $2.46 \pm 0.58$ g/dl
- Prealbumin $14.56 \pm 6.92$ mg/dl

Healing of Pressure Ulcers

145 consecutive pressure ulcers treated

What are the expected healing rates?

- **Diabetic foot ulcers** - 100% of diabetic foot ulcers will heal in absence of ischemia and osteomyelitis

- **Venous ulcers** - 100% healing of venous ulcers less than a year duration; 78% healing of venous ulcers greater than a year duration

- **Pressure Ulcer** - Prevent stage IV; 100% healing stages 2 and 3
Future: Can we make blood vessels grow in wounds? VEGF
Adenovirus Delivery System

Gene therapy using an adenovirus vector
Mechanical properties of healing wounds are measured by tensile strength.

Linear Incision: 30mm
Skin Strip: 30mm x 8mm

BKS.Cg-m +/+ Lepr db
Exp:41-24 DB
Day 14: 7/19/02

Local angiogenic VEGF therapy:
Skin mechanical testing

Test day: 21 days after wounding

Stiffness (N/mm)

VEGF  saline  vehicle

p=0.03  p=0.04
Human cell bank

Through partnership with Coriell Cell Bank. All cell cultures are grown and frozen in antibiotic-free media to aid in the detection and prevention of contamination.

Cultures are tested and found free of mycoplasma, bacteria, and fungi during expansion, at the time of frozen storage, and after recovery of stock for distribution from liquid nitrogen.
1. Molecular tools allow for purification and isolation of Epidermal Stem Cells from which one can recreate fully differentiated epidermis

How can we grow skin?

Morasso MI and Tomic-Canic, M. Biol Cell. 2005
97(3):173-83.
Opportunities

3. Epidermal stem cells (ESC) can be genetically engineered to sustain persistent expression of a transgene in tissue engineered skin.

Cell Repository Protocol

Quality Control of Cell Cultures

1. Receive blood
   - Retain 0.5 ml of blood in tube for microsatellite analysis
   - Remove aliquot for lymphocyte transformation
2. Separate lymphocytes and attempt transformation
3. Did cells transform?
   - Yes: Cryopreserve stock
   - No: Request resubmission
4. Cryopreserve stock
5. Recover and test second amnule

- Are cells viable?
  - Yes: Remove aliquot for mycoplasma testing
  - No: Discard culture
6. Test for mycoplasma contamination by PCR
7. Is culture free from mycoplasma contamination after recovery?
   - Yes: Perform microsatellite analysis on culture
   - No: Request resubmission
8. Did DNA fingerprint match that obtained from DNA retained in the tube?
   - Yes: Certify for NIGMS collection
   - No: Recover and test second amnule
   - No: Recover and test second amnule
   - No: Request resubmission
Human Skin Equivalent (HSE) Reconstructed in vitro from Normal Human Primary Keratinocytes and Fibroblasts Heals in a Similar Manner to Human Skin

- **24 hrs**
- **48 hrs**
- **72 hrs**

<table>
<thead>
<tr>
<th>HSE</th>
<th>Normal Human Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td></td>
</tr>
</tbody>
</table>
What is the Molecular Basis of Healing?
c-myc IS INDUCED IN NON HEALING WOUNDS

Untreated

Wound Healing Inhibitor GC

CHRONIC ULCER

Identification of the Chronic Wound Transcriptome
Bench to Bedside: From Microarray Analyses to Patient Biopsies

Gene Expression Profiles

Location A

Location B

Control

Location A

Location B

Location B

Location A
Cells from Location A do not Respond to WH Stimuli with VEGF

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>Control</th>
<th>Location A</th>
<th>Location B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>8</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td>24</td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>

% Wound (scratch) area

![Histogram](image13.png)

VEGF

- Control
- Location A
- Location B

0 hrs. 4 hrs. 8 hrs. 24 hrs.
Amphibian Limb Regeneration

Wound Healing (Days 1-2)

De-differentiation (Days 3-12)

Blastema formation (Days 13-21)

Re-differentiation & Pattern Formation (Days 22-40)
Limb Regeneration

Amphibians

Humans

?
Human Limb Regeneration

1. Healing Veterans’ wounds with current therapy
2. Education
3. Telemedicine
4. New Delivery systems: cells, antibiotics
5. Focus on developing tissue engineered components comparable to human tissue: Skin, blood vessels, neurons, cartilage, bone, muscles.