Using Animal Models to Help Solve Clinical Problems
- Overview
- Epidemiology
- Improving the Standards for Initial Wound Care
- Use of dual purpose bone grafts
- Developing a more relevant animal model
Research Cycle

Battlefield
Medical Problems

Data
Driven
Questions

Laboratory Research

Clinical Research
What are the problems?
Comparison of Statistics for Battle Casualties, 1941-2005

<table>
<thead>
<tr>
<th>Overall mortality of those wounded in combat</th>
<th>World War II</th>
<th>Vietnam War</th>
<th>Iraq &amp; Afghanistan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22.8%</td>
<td>16.5%</td>
<td>8.8%</td>
</tr>
</tbody>
</table>
1,566 soldiers sustained 6,609 combat wounds

3,575 were extremity wounds (82% of the soldiers had at least one extremity wound)

For every injured soldiers there are 2.3 extremity wounds
Extremity Injuries

- 53% of the extremity injuries were penetrating soft tissue wounds (1.2 for every injured Soldier)
- 26% were fractures (1 open fracture for every 2 injured Soldiers)
## Relationship to total number of wounds

### Operation Iraqi Freedom (OIF) U.S. Casualty Status *

**FATALITIES AS OF: September 14, 2009, 10 a.m. EDT**

<table>
<thead>
<tr>
<th>OIF U.S. Military Casualties by Phase</th>
<th>Total Deaths</th>
<th>KIA</th>
<th>Non-Hostile</th>
<th>WIA RTD **</th>
<th>WIA Not RTD **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combat Operations - 19 Mar 03 thru 30 Apr 03</td>
<td>139</td>
<td>109</td>
<td>30</td>
<td>116</td>
<td>429</td>
</tr>
<tr>
<td>Post Combat Ops - 1 May thru Present</td>
<td>4,194</td>
<td>3,355</td>
<td>839</td>
<td>17,517</td>
<td>13,432</td>
</tr>
<tr>
<td>OIF U.S. DoD Civilian Casualties</td>
<td>13</td>
<td>9</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>4,346</strong></td>
<td><strong>3,473</strong></td>
<td><strong>873</strong></td>
<td><strong>17,633</strong></td>
<td><strong>13,861</strong></td>
</tr>
</tbody>
</table>

### Operation Enduring Freedom (OEF) U.S. Casualty Status

**FATALITIES AS OF: September 14, 2009, 10 a.m. EDT**

<table>
<thead>
<tr>
<th>OEF U.S. Military Casualties</th>
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<th>Non-Hostile</th>
<th>WIA RTD **</th>
<th>WIA Not RTD **</th>
</tr>
</thead>
<tbody>
<tr>
<td>In and Around Afghanistan***</td>
<td>752</td>
<td>575</td>
<td>177</td>
<td>1,506</td>
<td>2,389</td>
</tr>
<tr>
<td>Other Locations****</td>
<td>69</td>
<td>3</td>
<td>66</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>OEF U.S. DoD Civilian Casualties</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Worldwide Total</strong></td>
<td><strong>822</strong></td>
<td><strong>579</strong></td>
<td><strong>243</strong></td>
<td><strong>1,506</strong></td>
<td><strong>2,390</strong></td>
</tr>
</tbody>
</table>

• 2.3 extremity injuries/wounded Soldier

= 37,377 extremity injuries

Total WIA Not RTD = 16,251
Injuries

E: Extremity,
H: Head/Neck,
A: Abdomen,
T: Thorax.

Extremities are the most commonly injured part of the body.
Extremity injury is responsible for most of the hospital costs.

Extremity injury is responsible for most of costs paid to soldiers for disabilities

The money paid out for disability is a good estimate of the amount of morbidity and functional loss of the soldier.
Injury severity relative to civilian medicine—Fractures

Type III – Either an open segmental fracture, and open fracture with extensive soft-tissue damage, or a traumatic amputation

1 Johnson, Burns et al. 2007
2 Gustilo and Anderson 1976
Type III tibial fractures

- All infected
- 37% delayed union associated with infection
- 14% require amputation
  - 80% due to infectious complication
Bottom Line

- Compared to previous wars
  - Likelihood of surviving war wounds has increased
  - Severity of wounds increased
- Multiple wounds-Polytrauma
- Penetrating soft-tissue trauma
- Fractures-Open/Type III
- Infection
Mission: Return to function
Research Areas

Infection
  - Prevention
  - Treatment
Repair/regeneration of tissue
  - Bone
  - Muscle
  - Skin
Improving the Standards for Initial Wound Care
Research Areas

Infection
  -Prevention
  -Treatment

Repair/regeneration of tissue
  -Bone
  -Muscle
  -Skin
Unanswered Questions

Irrigation
- Volume
- Method of delivery
- Optimal solution
- Timing

Debridement
- How much and how often

NPWT

Adjunctive Antibiotics

Silver bandages with NPWT

Reason for this research:
- Evidence based approaches are lacking
- Animal models of questionable validity
Irrigation Model

- Goat proximal tibial wound
- Thermal damage to muscle, periosteum, fascia
- Cortical bone defect
- Muscle crush
- 1 mL >$10^8$ inoculation

Pseudomonas aeruginosa (lux)

- Provides luminescence
- Proportional to bacterial quantity
- Correlation with quantitative cultures

![Diagram of the lux operon](image-url)
Photon Imaging

Highly sensitive
CCD camera
Pulse Lavage Imaging

Pre-Irrigation

Post-Irrigation
Mechanisms of Biofilm Tolerance
Antimicrobial Depletion

Slow Penetration  Stress Response  Altered Microenvironment  Persisters

Courtesy of Center for Biofilms Engineering
Ratio (Post/Pre)

<table>
<thead>
<tr>
<th></th>
<th>pre</th>
<th>post</th>
<th>48-hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>7.8±0.8</td>
<td>2.5±0.6</td>
<td>5.5±1.1</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>1.7±0.3</td>
<td>0.4±0.1</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>Benzalkonium Chloride</td>
<td>5.6±0.7</td>
<td>1.0±0.2</td>
<td>5.0±1.0</td>
</tr>
<tr>
<td>Castile Soap</td>
<td>5.5±0.9</td>
<td>0.8±0.4</td>
<td>5.7±1.7</td>
</tr>
</tbody>
</table>

Selected as a Highlight Paper from OTA Annual Meeting

<table>
<thead>
<tr>
<th></th>
<th>Pulsatile</th>
<th>post</th>
<th>48-hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulsatile</td>
<td>6.4 ± 3.3</td>
<td>1.9 ± 1.6</td>
<td>4.6 ± 2.9</td>
</tr>
<tr>
<td>Bulb Syringe</td>
<td>5.9 ± 2.6</td>
<td>1.5 ± 1.1</td>
<td>2.4 ± 1.3</td>
</tr>
</tbody>
</table>

Ratio (Post/Pre)

Post Irrigation

48 Hours Post Injury/Inoculation

- Pulsatile Lavage
- Bulb Syringe
Bulb Syringe Irrigation

6-Hr Pre-Tx  

6-Hr Post-Tx  

48-Hr
Pulse Lavage Irrigation

6-Hr Pre-Tx

6-Hr Post-Tx

48-Hr
Two days after debridement and irrigation

Bulb syringe

Pulsatile Lavage
High Pressure Parallel Flow

- High velocity jet of sterile saline creates vacuum
- Tissue is drawn in and excised

Versajet®, Smith and Nephew, Memphis, TN
<table>
<thead>
<tr>
<th></th>
<th>Pre 6 Hr</th>
<th>Post 6 Hr</th>
<th>Pre 48 Hr</th>
<th>Post 48 Hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulb</td>
<td>6.6±1.0</td>
<td>1.3±0.2</td>
<td>2.2±0.3</td>
<td>1.9±0.3</td>
</tr>
<tr>
<td>VersaJet</td>
<td>6.8±1.1</td>
<td>0.8±0.3</td>
<td>3.7±1.5</td>
<td>2.2±0.8</td>
</tr>
</tbody>
</table>

**Graph Description:**
- **Y-axis:** Ratio (Post/Pre)
- **X-axis:**
  - 6 Hours Pre-Irrigation
  - 6 Hours Post-Irrigation
  - 48 Hours Pre-Irrigation
  - 48 Hours Post-Irrigation
- **Legend:**
  - Red: Bulb Syringe
  - Blue: VersaJet

**Graph Note:**
- The graph illustrates the ratio changes over time for Bulb Syringe and VersaJet. The ratios are compared between pre- and post-irrigation periods, with error bars indicating variability.
- The data shows a decrease in ratio post-irrigation for both tools, with VersaJet showing a slightly lower ratio compared to Bulb Syringe in the post-irrigation period.
Adjunctive Therapy

Ratio (Post/Pre)

6 Hours Pre-Irrigation
6 Hours Post-Irrigation
48 Hours

CaSO4 Pellets
CaSO4 + Amikacin Pellets
PMMA + Amikacin Beads
Chitosan Sponge
Antibiotic Loaded Chitosan Sponge
Bacterial Quantity of *P. aeurginosa* in an Open Fracture Model

**Ratio (Post/Pre)**

- **No Treatment**
- **Chitosan**

Hours:
- 6 pre
- 6 post
- 48 pre

**P < 0.05**
Bacterial Quantity of *S. aureus* in an Open Fracture Model

Ratio (Post/Pre)

<table>
<thead>
<tr>
<th>Hours</th>
<th>No Treatment</th>
<th>Chitosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 pre</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>6 post</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>48 pre</td>
<td>0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

P < 0.05
Results

*P. aeruginosa* (6 hr)

**Control Group**

**Baseline (6 hr pre)**

**Treatment Group**

**Baseline (6 hr pre)**
Results

*P. aeruginosa* (48 hr)

Control Group

Final (48 hr)

Treatment Group

Final (48 hr)
Results
*S. aureus* (6 hr)

Control Group

Baseline (6 hr pre)

Treatment Group

Baseline (6 hr pre)
Results
*S. aureus* (48 hr)

Control Group

Treatment Group

Final (48 hr)

Final (48 hr)
Future Work

- Antimicrobials
Negative Pressure Wound Therapy
Modify existing model
HIPPA is practiced here, too!
Bacterial Quantity in Open Fracture Model

Percent of Baseline Value

WTD
NPWT with Sponge
NPWT with Gauze

0 100 200 300 400 500 600 700 800

6hr Pre 6hr Post 2day Pre 2day Post 4day Pre 4day Post 6day Pre

Percent of Baseline Value

WTD
NPWT with Sponge
NPWT with Gauze

0 100 200 300 400 500 600 700 800

6hr Pre 6hr Post 2day Pre 2day Post 4day Pre 4day Post 6day Pre
NPWT

Wet-to-Dry
Bacterial Quantity in Open Fracture Model

Percent of Baseline Value

- WTD
- NPWT with Sponge
- NPWT with Gauze
- NPWT with Silver Dressing

<table>
<thead>
<tr>
<th></th>
<th>6hr Pre</th>
<th>6hr Post</th>
<th>48hr Pre</th>
<th>48hr Post</th>
<th>96hr Pre</th>
<th>96hr Post</th>
<th>144hr Pre</th>
</tr>
</thead>
<tbody>
<tr>
<td>WTD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NPWT with Sponge</td>
<td></td>
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<td></td>
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<tr>
<td>NPWT with Silver Dressing</td>
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<td></td>
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</tr>
</tbody>
</table>

Percent of Baseline Value
What about Staph?
Can you use local antibiotics with NPWT?
Bead Pouch

6 hour pre

48 hour
NPWT with Beads

6 hour pre

Beads in place before photon imaging at 48 hours

48 hour with beads in place

48 hour with beads removed
What does this all mean?
Research Areas

Infection
  - Prevention
  - Treatment
Repair/regeneration of tissue
  - Bone
  - Muscle
  - Skin
Overview of Bone Regeneration
Extremity Injuries

- 53% of the extremity injuries were penetrating soft tissue wounds (1.2 for every injured soldier)
- 26% were fractures (1 open fracture for every 2 injured soldiers)
Tissue Engineering Components

Osteoconductive: Scaffold

Osteoinductive: Growth Factors

Osteogenic: Progenitor Cells

Ideal Scaffold
Staged Approach
ACTUAL Ideal Implant

Osteoconductive: Scaffold

Osteoinductive: Growth Factors

Osteogenic: Progenitor Cells

antibiotic
Dual Delivery Implant
Development of PUR scaffold for delivery of BMP and Antibiotic

BMP release profile from PUR scaffold

Vancomycin release profile from PUR scaffold
Rat Model

- 6-mm segmental defect in rat femur
- Stabilized with plate
Developing a More Stringent Animal Model for Evaluating Bone Grafts
Problem

- 32% of lower extremity limb salvages developed non-union
- Current standard of care (autograft) heals virtually every bone defect in animal models
  - CSDs
- The current animal models do not reflect the clinical scenario
  - Current animal models are not stringent enough.
- **This creates a glass ceiling** where we can not distinguish between fair, good, and great.
What we know

- Larger defects do not heal as well
- Soft tissue injury reduces the ability of fractures to heal
Potential Solution

- Large 5-cm defect in tibia of goats
- Concomitant stripping of periosteum and removal/injury to soft tissue envelope

Goal is to create a wound where autografts do not heal every time!
5-cm defect in tibia with interlocking nail
CT images of defect site

CT images of tibia. A) sagittal, false color of muscle, B) sagittal, C) coronal, D) transaxial view

Tibialis anterior

Gastrocnemius
Available autograft sites

Volumes of cancellous bones.
A, Humerus, C, Pelvis and femur. B, D show soft tissue. Bar, 10cm
Testing Platform

- Goal is to be able to determine which currently available and used bone grafts is best.
- Then, we will use this to evaluate emerging therapies.
Research Areas

Infection
- Prevention
- Treatment

Repair/regeneration of tissue
- Bone
- Muscle
- Skin
Questions?