Disclosures

• No personal disclosures pertaining to this presentation.

• The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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Extremity Trauma/Regenerative Medicine

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• Christina Hylden, MD
• Travis Burns, MD

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• George Christ, PhD (WFIRM)
• Roger Farrar, PhD (UT Austin)
Outline

• Background
  • Clinical problem
  • Review of muscle and muscle injury

• Current solutions for treating VML

• Tissue engineering and regenerative medicine (TE/RM)-current strategies

• TE/RM and rehabilitation

• Treatment of the remaining muscle mass

• The State of the Science
Comparison of Statistics for Battle Casualties, 1941-2005

- Mortality rate has decreased$^1$
- Severity of injuries has increased$^1$
- Polytrauma (4.3/wounded)

Impact of Military Trauma Care and Research – More live saves = more severe injuries

- Fatality Rate
- Injury Severity

Graph showing the Case Fatality Rate and Injury Severity Score from 2005 to 2013, with a decrease in Case Fatality Rate and increase in Injury Severity Score over time.
Extremities = Majority of all Injuries

Majority of Extremity Injuries = Soft-tissue and Fractures

Extremity Injuries = Majority of Healthcare Costs

**FIGURE 1.** Distribution of injuries, resources, and disability costs by body region. A, abdomen; E, extremity; H, head/neck; T, thorax.

Majority of Medical Retirements due to Extremity Injuries

Unfitting Conditions (n = 405)

- Extremity: 69%
- Thorax: 16%
- Abdomen: 3%
- Head: 3%
- Psych: 1%

1Cross et al., Battlefield Orthopaedic Injuries Cause the Majority of Long Term Disabilities. J AAOS 2012
Rank by count of unfitting conditions

Definition:
Muscle condition-Loss of muscle mass causing weakness

<table>
<thead>
<tr>
<th>Rank No.</th>
<th>Unfitting Condition</th>
<th>Frequency</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Degenerative arthritis</td>
<td>135</td>
</tr>
<tr>
<td>2</td>
<td>Nerve: Loss of function</td>
<td>102</td>
</tr>
<tr>
<td>3</td>
<td>Posttraumatic stress disorder</td>
<td>101</td>
</tr>
<tr>
<td>4</td>
<td>Pain</td>
<td>59</td>
</tr>
<tr>
<td>5</td>
<td>Lower extremity amputation</td>
<td>56</td>
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<td>6</td>
<td>Back pain</td>
<td>54</td>
</tr>
<tr>
<td>7</td>
<td>Eye condition</td>
<td>45</td>
</tr>
<tr>
<td>8</td>
<td>Traumatic brain injury</td>
<td>43</td>
</tr>
<tr>
<td>9</td>
<td>Hand condition</td>
<td>42</td>
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<tr>
<td>10</td>
<td>Muscle condition</td>
<td>39</td>
</tr>
<tr>
<td>11</td>
<td>Extremity scarring</td>
<td>35</td>
</tr>
</tbody>
</table>
Muscle Conditions

Ischemia-Reperfusion (I-R)
- Vascular injury, tourniquet
- Compartment Syndrome
- ECM intact-loss of cellular components

Volumetric Muscle Loss (VML)
- Physical loss of muscle tissue
- Loss of all components
Basic Anatomy of Skeletal Muscle

Extracellular Matrix (ECM)
Ultrastucture of Muscle Fiber

- Sarcomere
- Z line
- Thick filaments
- Thin filaments
- I band
- A band
- H zone
- Mitochondria
- Myofibril
- Sarcoplasmic reticulum
- Sarcolemma
- Thick filament
- Thin filament

[Diagram showing the structure of a muscle fiber with labeled parts]
Relationship of Muscle Fiber and ECM

Role of ECM
- Structural support (myofibrils, blood vessels, nerves)
- Passive elasticity
- Lateral force transmission
- Growth factors
- Regulates alignment of cells
Repair and Regeneration

Muscle Injury: General

Successful Regeneration

Muscle regeneration

Activation of satellite cells

Tissue Repair Phases and Timescale

Fibrotic Scar

Healthy muscle

Muscle damage

Muscle degeneration

Muscle fibrosis

Myofiber necrosis

Connective tissue activation

Inflammatory reaction

Cytokines

Bleeding

Inflammation

Proliferation

Remodelling

Hours
Days
Weeks
Months
Volumetric Muscle Loss (VML)

The traumatic or surgical loss of skeletal muscle with resultant functional impairment. (Grogan and Hsu 2011)
Current Solutions

• Muscle flap
• Functional muscle transfer
• Rehabilitation
Muscle Flap

Goal:
- Bone coverage
- Not muscle function
Free Functioning Muscle Transfer
Lin, Lin et al. 2007

Typically limited to upper extremity
Complex
Donor site morbidity
Current Solutions

• Muscle flap
• Functional muscle transfer
• Rehabilitation
• Tissue Engineering and Regenerative Medicine
Current TE strategies for VML

• Bioreactor Based Approach (Christ)
  • Acellular scaffold + cells ± cyclic stretch
  • Application-small muscles
Implantation of *In Vitro* Tissue Engineered Muscle Repair Constructs and Bladder Acellular Matrices Partially Restore *In Vivo* Skeletal Muscle Function in a Rat Model of Volumetric Muscle Loss Injury

Benjamin T. Corona, PhD,1,2 Catherine L. Ward, PhD,1,2 Hannah B. Baker, BS,1 Thomas J. Walters, PhD,2 and George J. Christ, PhD1

BAM ± MDSCs

BAM-Bladder Acellular Matrix
MDSC-Muscle Derived Stem Cells
Current TE strategies for VML

- Accellular Approach (Badylak)

- Constructive Remodeling (Small Intestinal Submucosa (SIS) Extracellular Matrix FDA approved)
Clinical Application of an Acellular Biologic Scaffold for Surgical Repair of a Large, Traumatic Quadriceps Femoris Muscle Defect

by Vincent J. Mase, MD; Joseph R. Hsu, MD; Steven E. Wolf, MD; Joseph C. Wenke, PhD; David G. Baer, PhD; Johnny Owens, MPT; Stephen F. Bedylak, DVM, PhD, MD; Thomas J. Walters, PhD

An Acellular Biologic Scaffold Promotes Skeletal Muscle Formation in Mice and Humans with Volumetric Muscle Loss

Brian M. Sicari,1,2* J. Peter Rubin,1,3,4* Christopher L. Dearth,1,2 Matthew T. Wolf,1,4 Fabrisia Ambrosio,1,5 Michael Boninger,1,4,5 Neill J. Turner,1,2 Douglas J. Weber,4,5 Tyler W. Simpson,5 Aaron Wyse,6 Elke H. P. Brown,5 Jenna L. Dziki,1,4 Lee E. Fisher,5 Spencer Brown,1,3 Stephen F. Badylak1,2†

Table 1. Patient information. Relevant information from each patient (n = 5) in the present study. Tissue deficit estimated from MRI or CT scan. IED, improvised explosive device.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Injury site (side)</th>
<th>Cause of injury</th>
<th>Time since injury (months)</th>
<th>Previous surgeries</th>
<th>Tissue deficit (estimate)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>34</td>
<td>Anterior tibial compartment (left)</td>
<td>Exercise-induced</td>
<td>13</td>
<td>5</td>
<td>58%</td>
<td>Military</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>37</td>
<td>Anterior tibial compartment (left)</td>
<td>Skiing accident</td>
<td>32</td>
<td>4</td>
<td>67%</td>
<td>Civilian</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>28</td>
<td>Quadriceps (left)</td>
<td>IED blast</td>
<td>18</td>
<td>14</td>
<td>68%</td>
<td>Military</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>27</td>
<td>Quadriceps (right)</td>
<td>IED blast</td>
<td>89</td>
<td>50</td>
<td>83%</td>
<td>Military</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>32</td>
<td>Anterior/lateral tibial compartment (left)</td>
<td>Skiing accident</td>
<td>85</td>
<td>8</td>
<td>90%</td>
<td>Civilian</td>
</tr>
</tbody>
</table>
Table 3. Force production data from each patient. Strength measures as assessed with a handheld dynamometer from each patient. Data are percent change from presurgical maximum obtained after physical therapy.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Activity</th>
<th>6–8 weeks postsurgical (%)</th>
<th>10–12 weeks postsurgical (%)</th>
<th>24–28 weeks postsurgical (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Dorsiflexion</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>Dorsiflexion</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>Knee extension</td>
<td>−10.0</td>
<td>18.3</td>
<td>20.0</td>
</tr>
<tr>
<td>4</td>
<td>Knee extension</td>
<td>127.9</td>
<td>149.2</td>
<td>136.1</td>
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<tr>
<td>5</td>
<td>Dorsiflexion</td>
<td>−33.3</td>
<td>16.7</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Average | 16.92 | 36.84 | 37.88 |
SEM      | 28.4  | 28.4  | 25.3  |
Current TE strategies for VML

- Accellular + Cellular (Walters)
  - Preplaced Biological scaffold
  - Exogenous cell source
  - Body is the bioreactor
A Standardized Rat Model of Volumetric Muscle Loss Injury for the Development of Tissue Engineering Therapies

Xianwu Wu1,2 Benjamin T. Connor1 Xianwu Chen1,3 and Thomas J. Walters1

VOLUMETRIC MUSCLE LOSS MODEL

FIG. 1. Illustration of rat tibialis anterior (TA) muscle surgical procedure. Approximately 20% of the rat TA muscle was excised to create an endogenously irrecoverable VML injury. See Materials and Methods for a description of images.
VML injury results in 30% force reduction

Rat “Biodex”
The promotion of a functional fibrosis in skeletal muscle with volumetric muscle loss injury following the transplantation of muscle-ECM

Benjamin T. Corona, Xiaowu Wu, Catherine L. Ward, Jennifer S. McDaniel, Christopher R. Rathbone, Thomas J. Walters

US Army Institute of Surgical Research, Extremity Trauma and Regenerative Medicine, 3698 Chambers Pass, Fort Sam Houston, TX 78234, USA
VML repair w/ mECM improves function

A

Isometric Force
(N/kg body wt)

B

Time (S)

Peak Tetanic Isometric Force
(N/kg body wt)

Uninj. NR mECM
VML repair w/ BMSCs don’t improve function
VML repair w/ mECM ± BMSCs does not result in new muscle formation

- ECM
- ECM+BMSC

No new muscle here
VML repair w/ mECM results less damage to remaining muscle

Non-repaired

Repaired
VML repair w/ mECM results less damage to remaining muscle

Injury radiate out from wound

Non-repaired

Repaired
VML repair w/ mECM results less damage to remaining muscle
VML results in chronic myopathy

16 weeks post-injury

Fibrosis and chronic inflammation

Note: Central nuclei
VML repair w/ mECM results in fibrotic scar
Proposed Mechanism

- Improves longitudinal force transmission
- Reduces strain injury in RMM
- Improved quality of muscle = better ability to rehabilitate?
The promotion of a functional fibrosis in skeletal muscle with volumetric muscle loss injury following the transplantation of muscle-ECM

Benjamin T. Corona, Xiaowu Wu, Catherine L. Ward, Jennifer S. McDaniel, Christopher R. Rathbone, Thomas J. Walters*
The promotion of a functional fibrosis in skeletal muscle with volumetric muscle loss injury following the transplantation of muscle-ECM

Benjamin T. Corona, Xiaowu Wu, Catherine L. Ward, Jennifer S. McDaniel, Christopher R. Rathbone, Thomas J. Walters* 
US Army Institute of Surgical Research, Department of Trauma and Regenerative Medicine, JBSA, Lackland, San Antonio, TX 78236 USA
Recap to this point

- Repair w/ ECM improves function
- ECM = ECM+BMSC
- Repair w/ ECM ± BMSC = no significant muscle regeneration
- VML injury results in long-term myopathy in remaining muscle
- Repair w/ ECM results in fibrotic scar at wound site
  - Improves force transmission
  - Protects remaining muscle mass for myopathic condition
Recap to this point

- Repair w/ ECM improves function
- ECM = ECM+BMSC
- Repair w/ ECM ± BMSC = no significant muscle regeneration
- VML injury results in long-term myopathy in remaining muscle
- Repair w/ ECM results in fibrotic scar at wound site
  - Improves force transmission
  - Protects remaining muscle mass for myopathic condition
- Muscle regeneration requires myogenic cell source
Myogenic cell sources

- **Satellite Cells**
  - Require expansion to obtain enough
  - Regulatory issues
  - Poor survival when transplanted

- **Single muscle fibers**
  - Regulatory issues
  - Technically difficult

- **Minced muscle autograft**
  - No regulatory issues
  - Satellite cells + muscle progenitor cells + endothelial progenitor cells + ECM
  - Proven regenerative capacity (Carlson et al, 1968; Studitsky, 1964)
Autologous minced muscle grafts: a tissue engineering therapy for the volumetric loss of skeletal muscle


Extremity Trauma and Regenerative Medicine Research Program, United States Army Institute of Surgical Research, Fort Sam Houston, Texas

Submitted 21 June 2013; accepted in final form 20 July 2013
Dramatic muscle regeneration

Fig. 5. Minced graft transplants promote de novo skeletal muscle fiber regeneration in VML-injured muscle. A: whole TA muscle cross sections from uninjured and VML-injured NR and MG-repaired muscles 16 wk after injury are presented. B–E: TA muscle cross sections probed for myosin (MF20) and collagen I were qualitatively analyzed for tissue regeneration in the defect area. Muscles were harvested from NR (B) and MG-repaired muscles at 8 (C) and 16 wk (D and E) postinjury. Four to six muscles per group and time were analyzed. Note: diagram of the TA muscle cross section illustrates where the depicted images for each group were captured. White dotted lines denote the approximate interface between the remaining muscle mass (left) and the defect area (right). Scale bars = 200 μm.
Regenerated muscle is innervated
Running further improves VML repair with MG

A

![Graph showing changes in VML & MG Repair with weeks (post-injury).]

Max Torque

- Uninj
- No Repair
- MG Sed
- MG Run

Max Torque

- Nm/mg body wt

B

C

D

E

Laminin

Myosin/ Collagen 1

Sed

Run

F

G

H

I

J

K

Fiber Area (μm²)

Min. Diameter (μm)

Circularity

Myosin (% Area)

Col. 1 (% Area)

Col. 1 Expression

Injured : Contralateral
Study Summary

- Minced autograft:
  - Improves muscle function
  - regenerates new innervated muscle
- Limitation:
  - Donor site morbidity

50% Less Donor
Minced Muscle Tissue
Achieves Comparable
Functional Restitution
as a 100% Replacement
VML injury – impact on remaining muscle
Impact of rehab following VML

Graphs showing the impact of rehabilitation following a Virtual Muscle Loss (VML) condition. Graph A illustrates the increase in distance traveled (km/week) over weeks post-injury, with a peak around week 5. Graph B shows the body weight (grams) over weeks post-injury, with a significant decrease following injury and a recovery phase with elevated body weight. The graphs compare the activity levels (SED vs. RUN) with activity starting at different points post-injury.
Muscle function improves with rehab

Torque-Frequency
(Nmm/g body wt)

Frequency (Hz)

A.

T_{\text{max}} \text{ (injured/uninjured)}

B.

Absolute (Nmm)

20% improvement

C.

Relative to TA wt. (%)

15% improvement

RUN

SED

RUN

SED
Mechanism

- Total number of fibers-no diff (data not shown)
- Size of fibers-no diff, i.e., no hypertrophy
Early rehab exacerbates myopathic condition.
Early rehab does not exacerbate fibrosis
Resistance exercise required for hypertrophy

The problem:
- Heavy loads = increased shear stress
- Limited by pain

Blood Flow Restricted Exercise
- Low resistance (20-30% 1RM)
- Well tolerated - Low pain
- Dramatic improvements in force

Blood Flow Restriction Rehabilitation for Extremity Weakness: A Case Series
Hylden, Christina, MD, San Antonio Military Medical Center
Burns, Travis, MD, San Antonio Military Medical Center
Stinner, Daniel, MD, United States Army Institute of Surgical Research
Owens, Johnny, BS, MPT, Center for the Intrepid

Figure 2. Mean change in peak torque (Nm), power (W), and work (J) for all seven patients in case series. Speed 1 is 90 degrees/second. Speed 2 is 300 degrees/second.
Muscle Injury: Fibrosis = increased muscle stiffness

Increased stiffness
- Decreased ROM
- Increased susceptibility to injury
Can blocking TGF-β1 reduce fibrosis?

**Figure 2** Illustration of the TGF-β1 signaling pathways and the mechanism of therapeutics.
Losartan blocks collagen but…

Statistical Analysis: One Way Anova, p=<0.05*, Data = Mean ± SEM
...reduces muscle regeneration and function

Increased intramuscular collagen necessary to protect muscle
What is the State of the Science?

- Current treatment options are limited
- Repair with ECM results in improved function
- Exogenous cell source required for muscle regeneration
- Minced muscle offers an easy treatment option for delivering ECM and exogenous cells
- Mechanical activity improves outcome VML repair
- VML injury not limited to wound-remaining muscle undergoes long-term myopathy
- Rehab research required to determine most effective way of improving function of remaining muscle
Design
Improving vascularization with microvascular fragments - Rathbone

Day 7

Lectin – Vessels  DAPI – Nuclei  GFP – implant

GFP – implant

Vessel Density (vessels/grid intersection/mm²)

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
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