The Biomaterial Gap in Tissue Engineering can be addressed and applied to Combat Casualty Care

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Disclosures and Notices

Luke Burnett is a board member, officer and shareholder of KeraNetics Inc.

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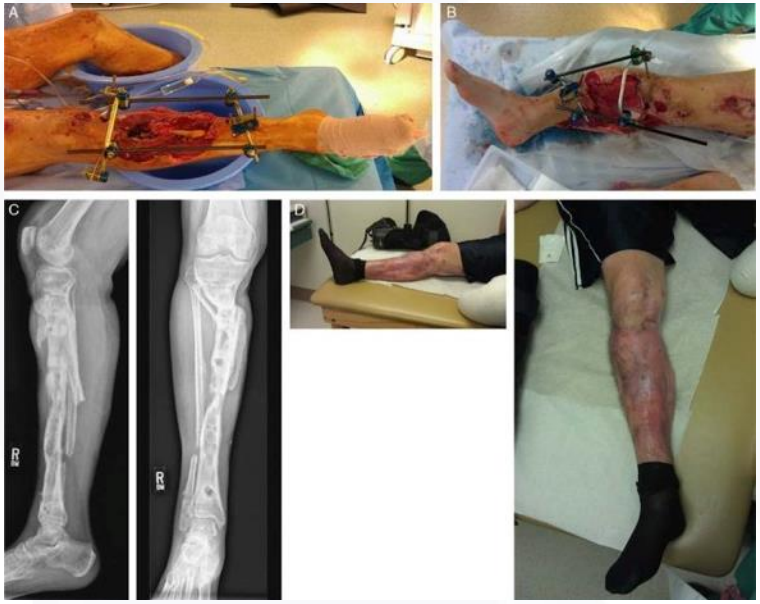
Battlefield Injuries Are More Complex

Mechanism of Injury – US Wars

	GSW	Explosion
Civil War	91%	9%
WWI	65%	35%
WWII	27%	73%
Korea	31%	69%
Vietnam	35%	65%
Iraq/Afghan	19%	81%

Owens et. al J Trauma 2008



Elston et. al Curr Orthop Pract 2013

My Vehicle Iraq 2004

My Vehicles Iraq 2008

Armor/Medical Care Has Improved:

- Severe injuries more survivable
- More extremity/bone trauma due to IEDs
- Transport times WILL INCREASE

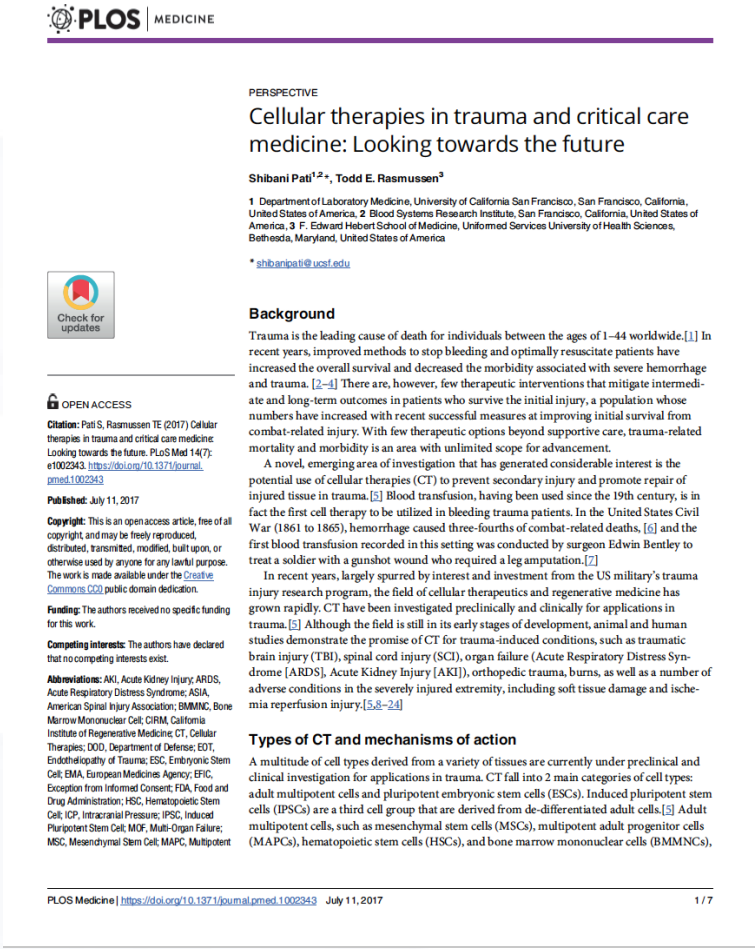
Solutions that were Role IV/V need to be pushed to Roll III and below

2035 Challenge for Battlefield Treatment

Challenge: Peer/Near Peer Conflicts, or Pacific theater will extend or even prevent transport

One potential solution:

- **Stop fighting the last war**
- **Expand the concept and capabilities of prolonged field care**
- **Role IV/V based solutions (such as Regenerative Medicine) can be pushed to Role III and below**



PLOS | MEDICINE

PERSPECTIVE

Cellular therapies in trauma and critical care medicine: Looking towards the future

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Check for updates

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Abbreviations: AKI, Acute Kidney Injury; ARDS, Acute Respiratory Distress Syndrome; ASIA, American Spinal Injury Association; BMMNC, Bone Marrow Mononuclear Cell; CIRM, California Institute of Regenerative Medicine; CT, Cellular Therapies; DOD, Department of Defense; EOT, Endotheliopathy of Trauma; ESC, Embryonic Stem Cell; EMA, European Medicines Agency; EFIC, Exception from Informed Consent; FDA, Food and Drug Administration; HSC, Hematopoietic Stem Cell; ICP, Intracranial Pressure; IPSC, Induced Pluripotent Stem Cell; MOF, Multi-Organ Failure; MSC, Mesenchymal Stem Cell; MAPC, Multipotent

Background

Trauma is the leading cause of death for individuals between the ages of 1–44 worldwide.[1] In recent years, improved methods to stop bleeding and optimally resuscitate patients have increased the overall survival and decreased the morbidity associated with severe hemorrhage and trauma. [2–4] There are, however, few therapeutic interventions that mitigate intermediate and long-term outcomes in patients who survive the initial injury, a population whose numbers have increased with recent successful measures at improving initial survival from combat-related injury. With few therapeutic options beyond supportive care, trauma-related mortality and morbidity is an area with unlimited scope for advancement.

A novel, emerging area of investigation that has generated considerable interest is the potential use of cellular therapies (CT) to prevent secondary injury and promote repair of injured tissue in trauma.[5] Blood transfusion, having been used since the 19th century, is in fact the first cell therapy to be utilized in bleeding trauma patients. In the United States Civil War (1861 to 1865), hemorrhage caused three-fourths of combat-related deaths, [6] and the first blood transfusion recorded in this setting was conducted by surgeon Edwin Bentley to treat a soldier with a gunshot wound who required a leg amputation.[7]

In recent years, largely spurred by interest and investment from the US military's trauma injury research program, the field of cellular therapeutics and regenerative medicine has grown rapidly. CT have been investigated preclinically and clinically for applications in trauma.[5] Although the field is still in its early stages of development, animal and human studies demonstrate the promise of CT for trauma-induced conditions, such as traumatic brain injury (TBI), spinal cord injury (SCI), organ failure (Acute Respiratory Distress Syndrome [ARDS], Acute Kidney Injury [AKI]), orthopedic trauma, burns, as well as a number of adverse conditions in the severely injured extremity, including soft tissue damage and ischemia reperfusion injury.[5,8–24]

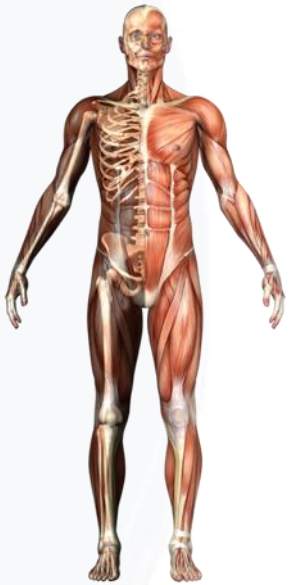
Types of CT and mechanisms of action

A multitude of cell types derived from a variety of tissues are currently under preclinical and clinical investigation for applications in trauma. CT fall into 2 main categories of cell types: adult multipotent cells and pluripotent embryonic stem cells (ESCs). Induced pluripotent stem cells (IPSCs) are a third cell group that are derived from de-differentiated adult cells.[5] Adult multipotent cells, such as mesenchymal stem cells (MSCs), multipotent adult progenitor cells (MAPCs), hematopoietic stem cells (HSCs), and bone marrow mononuclear cells (BMMNCs),

Regenerative Medicine: An Industry Perspective

Three major challenges in Regenerative Medicine

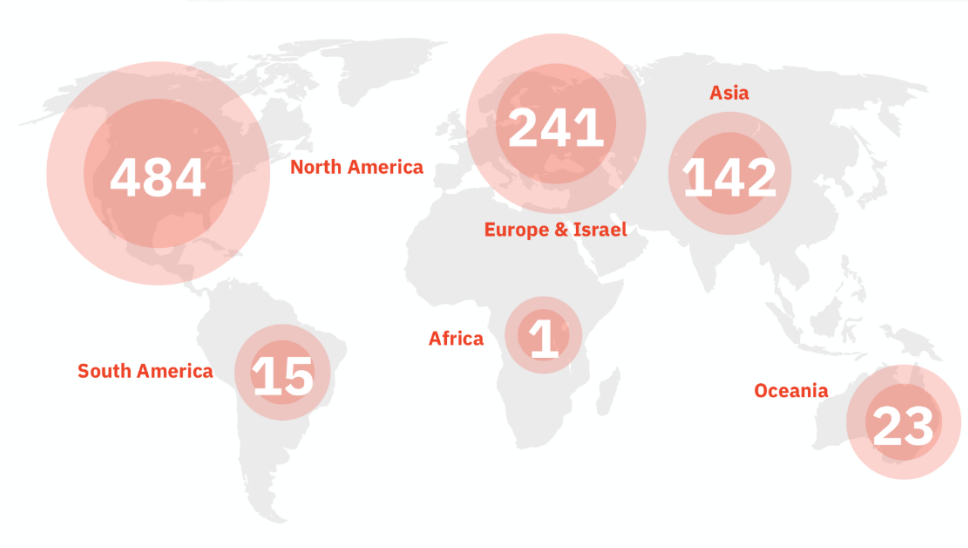
1. Technologies stove-piped as cell, material or biologic
2. Regulatory path can require combination of drug, device and biologic manufacturing controls
3. Researchers/Media over-interpret animal or clinical results which can produce unrealistic expectations



Has over hype damaged our field?

- **Can we create viable complex organs/tissues yet?**
- **Does the public think we can?**

“Regen Med” Worldwide Market



906 Regenerative Medicine companies as of 2018

$13B (USD) Financing deals in 2018

Total 2018 Global Financings

TOTAL GLOBAL FINANCING
$13.3 Billion
↑ 73%
Increase from 2017

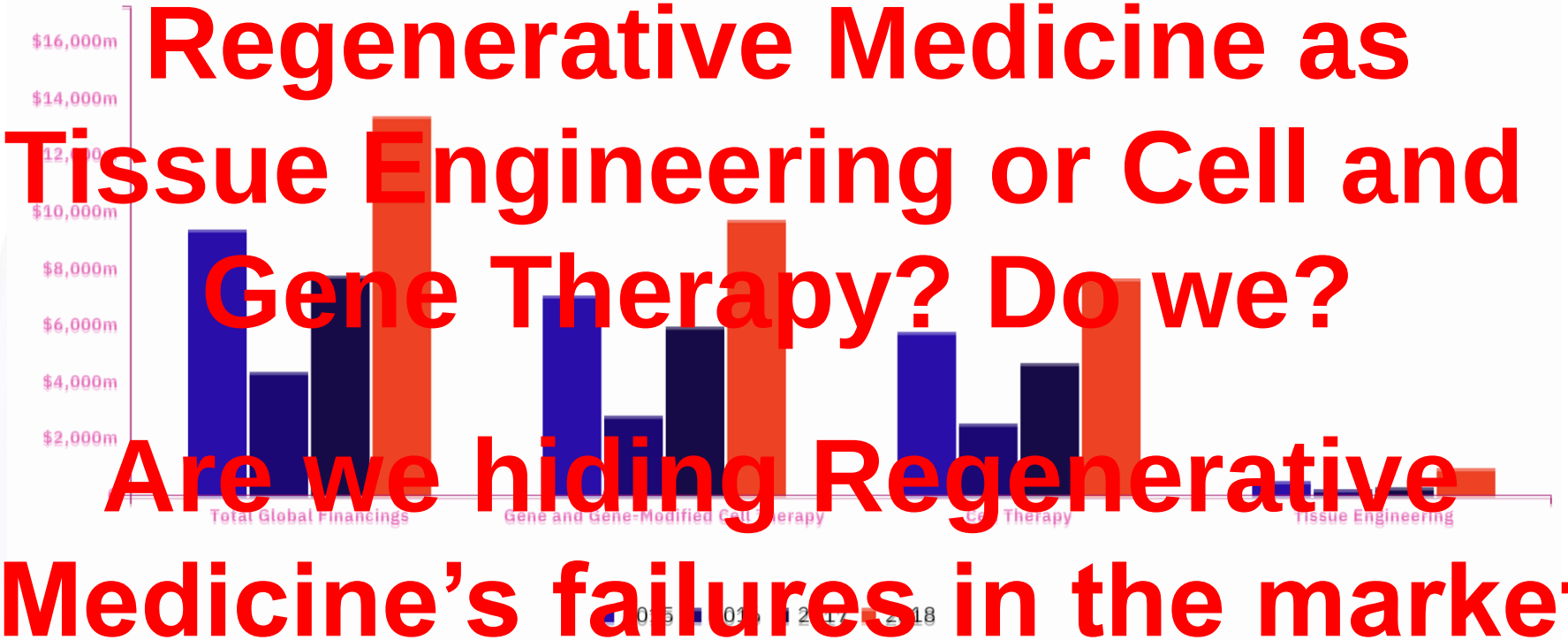
GENE & GENE-MODIFIED CELL THERAPY
$9.7 Billion
↑ 64%
Increase from 2017

CELL THERAPY
$7.6 Billion
↑ 64%
Increase from 2017

TISSUE ENGINEERING
$937 Million
↓ 258%
Decrease from 2017

“Regen Med” Global Financings 2015-18

Does the Public view Regenerative Medicine as Tissue Engineering or Cell and Gene Therapy? Do we?



Are we hiding Regenerative Medicine’s failures in the market with Cell and Gene Therapy?

Alliance for Regenerative Medicine 2018

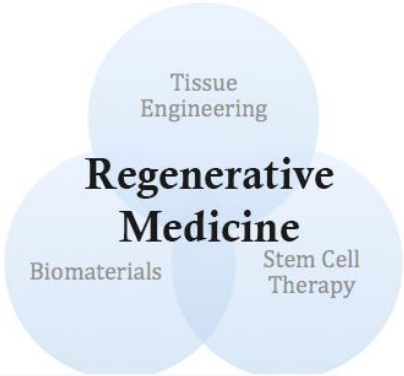
Tissue Engineering: A Divided House

2 Fields

- Cells vs. Biomaterials
  - Cells: “Magic bullets” – biologists/clinicians enamoured by pathways, VAST majority Regen Med clinical studies cell based
  - Biomaterials: “Matrix only” – engineers enamoured by porosity and biomechanics

We are both wrong!

- Cells are not magic
- Materials do not solve every problem
- Cell + Biomaterial ≠ Organ… yet



Are there short-term solutions to tissue regeneration that meet grant (academic) and investor (industry) timelines?

Is "Functional Fibrosis" the Best We Can Do?

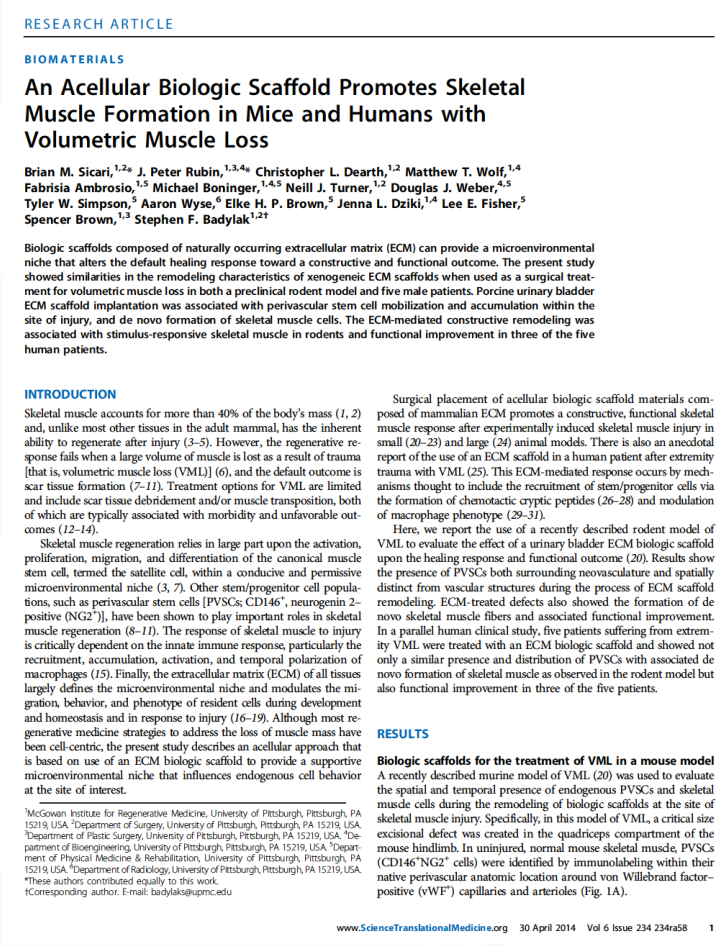
Dr. Badylak's Urinary Bladder Matrix Study

- 5 Patients – 58 – 90% deficit
  - 5-50 previous surgeries
  - 3 Military, 2 Civilian patients
  - MatriStem (UBM) implant
  - 6 month follow-up

- Patient #4

  136% improvement in knee extension

- All patients showed increases in angiogensis and desmin positive cells



RESEARCH ARTICLE

BIOMATERIALS

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

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Biologic scaffolds composed of naturally occurring extracellular matrix (ECM) can provide a microenvironmental niche that alters the default healing response toward a constructive and functional outcome. The present study showed similarities in the remodeling characteristics of xenogeneic ECM scaffolds when used as a surgical treatment for volumetric muscle loss in both a preclinical rodent model and five male patients. Porcine urinary bladder ECM scaffold implantation was associated with perivascular stem cell mobilization and accumulation within the site of injury, and de novo formation of skeletal muscle cells. The ECM-mediated constructive remodeling was associated with stimulus-responsive skeletal muscle in rodents and functional improvement in three of the five human patients.

INTRODUCTION

Skeletal muscle accounts for more than 40% of the body's mass (*1*, *2*) and, unlike most other tissues in the adult mammal, has the inherent ability to regenerate after injury (*3*–*5*). However, the regenerative response fails when a large volume of muscle is lost as a result of trauma [that is, volumetric muscle loss (VML)] (*6*), and the default outcome is scar tissue formation (*7*–*11*). Treatment options for VML are limited and include scar tissue debridement and/or muscle transposition, both of which are typically associated with morbidity and unfavorable outcomes (*12*–*14*).

Skeletal muscle regeneration relies in large part upon the activation, proliferation, migration, and differentiation of the canonical muscle stem cell, termed the satellite cell, within a conducive and permissive microenvironmental niche (*3*, *7*). Other stem/progenitor cell populations, such as perivascular stem cells [PVSCs; $CD146^+$, neurogenin 2–positive ($NG2^+$)], have been shown to play important roles in skeletal muscle regeneration (*8*–*11*). The response of skeletal muscle to injury is critically dependent on the innate immune response, particularly the recruitment, accumulation, activation, and temporal polarization of macrophages (*15*). Finally, the extracellular matrix (ECM) of all tissues largely defines the microenvironmental niche and modulates the migration, behavior, and phenotype of resident cells during development and homeostasis and in response to injury (*16*–*19*). Although most regenerative medicine strategies to address the loss of muscle mass have been cell-centric, the present study describes an acellular approach that is based on use of an ECM biologic scaffold to provide a supportive microenvironmental niche that influences endogenous cell behavior at the site of interest.

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Surgical placement of acellular biologic scaffold materials composed of mammalian ECM promotes a constructive, functional skeletal muscle response after experimentally induced skeletal muscle injury in small (*20*–*23*) and large (*24*) animal models. There is also an anecdotal report of the use of an ECM scaffold in a human patient after extremity trauma with VML (*25*). This ECM-mediated response occurs by mechanisms thought to include the recruitment of stem/progenitor cells via the formation of chemotactic cryptic peptides (*26*–*28*) and modulation of macrophage phenotype (*29*–*31*).

Here, we report the use of a recently described rodent model of VML to evaluate the effect of a urinary bladder ECM biologic scaffold upon the healing response and functional outcome (*20*). Results show the presence of PVSCs both surrounding neovasculature and spatially distinct from vascular structures during the process of ECM scaffold remodeling. ECM-treated defects also showed the formation of de novo skeletal muscle fibers and associated functional improvement. In a parallel human clinical study, five patients suffering from extremity VML were treated with an ECM biologic scaffold and showed not only a similar presence and distribution of PVSCs with associated de novo formation of skeletal muscle as observed in the rodent model but also functional improvement in three of the five patients.

RESULTS

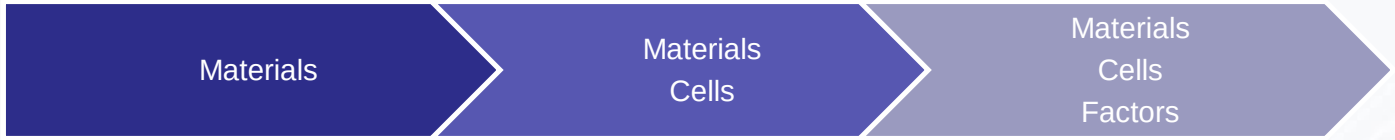
Biologic scaffolds for the treatment of VML in a mouse model

A recently described murine model of VML (*20*) was used to evaluate the spatial and temporal presence of endogenous PVSCs and skeletal muscle cells during the remodeling of biologic scaffolds at the site of skeletal muscle injury. Specifically, in this model of VML, a critical size excisional defect was created in the quadriceps compartment of the mouse hindlimb. In uninjured, normal mouse skeletal muscle, PVSCs ($CD146^+NG2^+$ cells) were identified by immunolabeling within their native perivascular anatomic location around von Willebrand factor–positive (vWF^+) capillaries and arterioles (Fig. 1A).

Sicari et al. Sci Translational Med 2014

Regen Med: A Stepwise Approach

- **Researchers MUST set realistic expectations**
  - Whole complex organ/tissue regeneration is NOT yet possible
    - Scientific challenges not yet solved
    - Lack of regulatory clarity for Regen Med combination product technologies a hurdle
  - Highlight beneficial science, not science fiction
  - Temper expectations for clinical research
- **Address the Gaps**
  - Extensive clinical progress on Cell based therapies
  - VERY few clinical studies conducted to test materials
- **Proposed Solution – The Crawl/Walk/Run Clinical Plan**

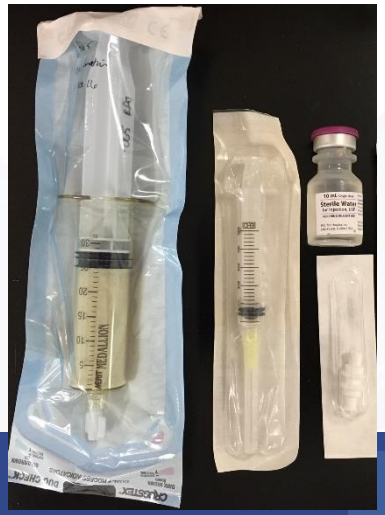


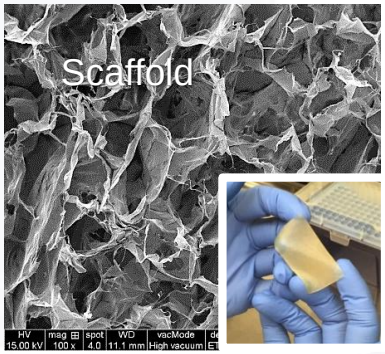
INCLUDE industry collaborators to generate market focused development plans

An Example Biomaterial: Keratin

Combines advantages of synthetic and biologic biomaterials:

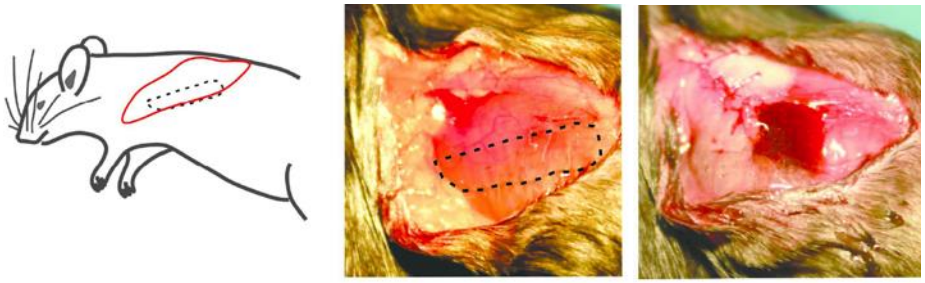
- **Human-Derived**
- **Safe Toxicity Profile (Biocompatible by ISO 10993)**
- **Excellent Cell Attachment and Proliferation Medium**
- **Tunable Degradation Rate**
- **Can Deliver Cells, Growth Factors, and Drugs**
- **Engineered Material Forms (liquid to solid)**
- **Manufactured Consistently in Large Quantities**
- **Naturally Remodeled by the Body**
- **No Mammalian Keratinases**
- **Can be Cross-Linked**





Muscle Defect Pre-Clinical Testing

Mouse Latissimus Dorsi Defect Model

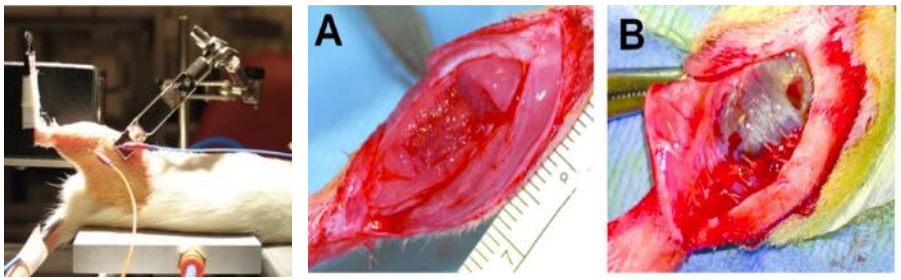


GROUPS	
No Repair (n=9)	Keratin + IGF + bFGF (n=8)
BAM (n=8)	Keratin + MPC (n=8)
Keratin (n=6)	Keratin + IGF + MPC (n=8)
Keratin + IGF (n=8)	Keratin + bFGF + MPC (n=9)
Keratin + bFGF (n=8)	Keratin + IGF + bFGF + MPC (n=8)

- **8 week time point**
- **Muscle force contraction**
- **Histology: H&E, Massons, Myosin**

Passipieri et al. Tissue Engineering 2017

Rat Tibialis Anterior Defect Model

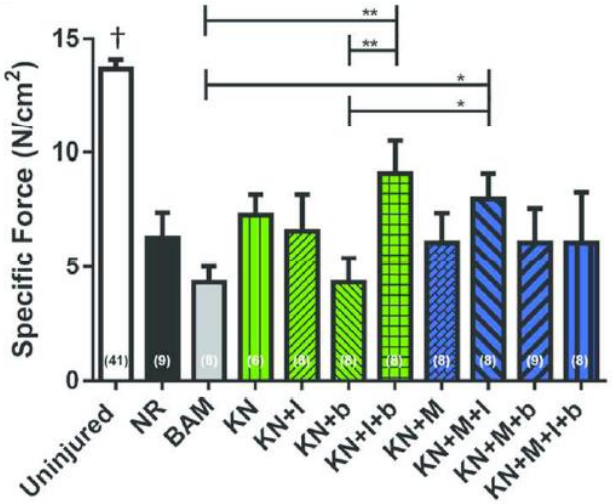


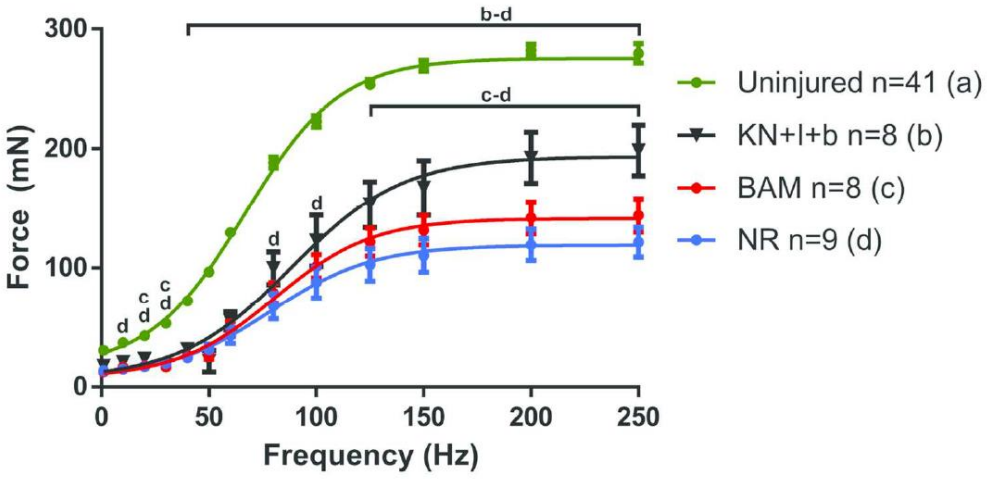
GROUPS	
8 Wk No Repair (n=7)	8 Wk Keratin + IGF + bFGF (n=8)
8 Wk BAM (n=8)	8 Wk Keratin + MPC (n=8)
8 Wk Keratin (n=6)	8 Wk Keratin + IGF + MPC (n=8)
8 Wk Keratin + IGF (n=8)	8 Wk Keratin + bFGF + MPC (n=9)
8 Wk Keratin + bFGF (n=8)	8 Wk Keratin + IGF + bFGF + MPC (n=8)
4-12 Wk No Repair (n=9)	4-12 Wk Keratin + IGF (n=8)
4-12 Wk BAM (n=8)	4-12 Wk Keratin + bFGF (n=8)
4-12 Wk Keratin (n=6)	4-12 Wk Keratin + IGF + bFGF (n=8)

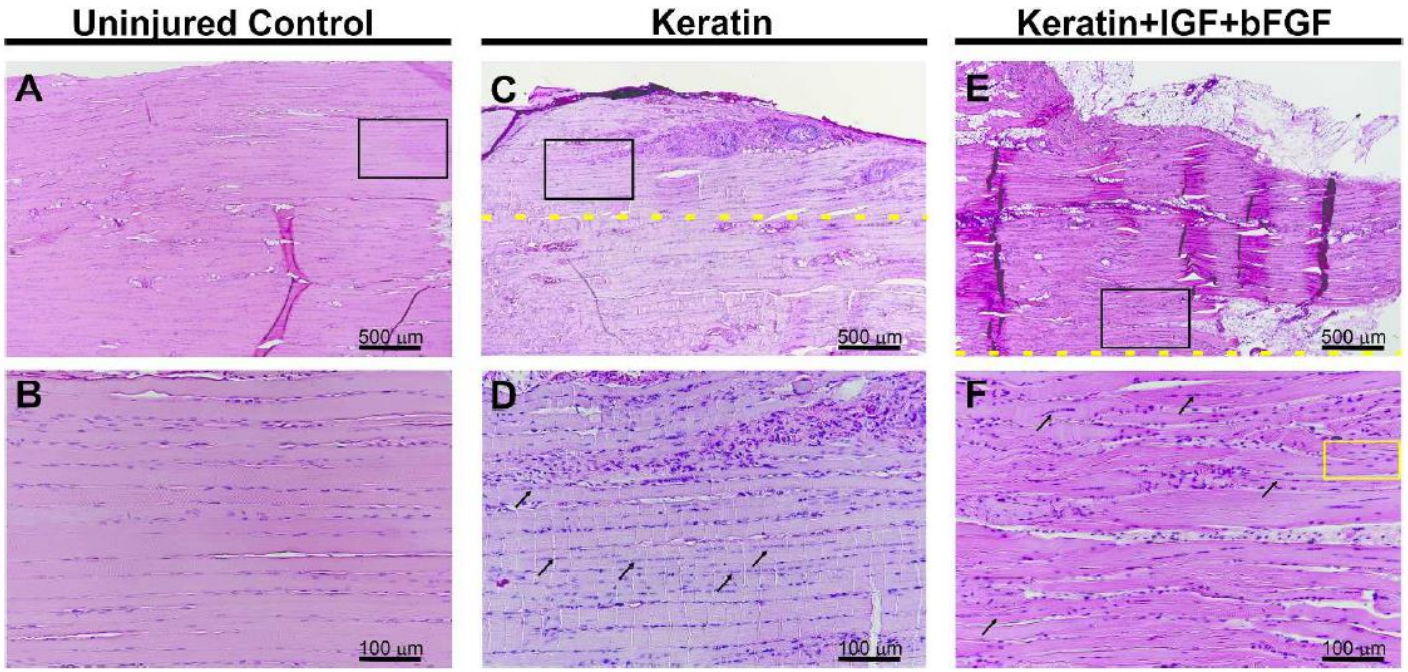
- **8 week with cells, 4 and 12 week time points without cells**

Baker et. al Tissue Engineering 2017

Keratin Matrix Improves Function







Baker et. al Tissue Engineering 2017

Commercialization Considerations

- Matrix Only

↑ market ↑ regulatory path ↓ reimbursement

- Matrix Plus Cells

↑ market ↓ regulatory path ↑ reimbursement

- Matrix Plus Cells Plus Growth Factors

↑ market ↓ regulatory path ↑ reimbursement

Regulatory pathway for Regen Med technologies are complex and involve SIGNIFICANT costs

Creates a significant barrier for new TE technologies

Conclusions

Summary

- Future conflicts will likely require redefining prolonged field care
- Future combat theaters will need solutions that can regenerate and repair soft tissue defects and volumetric muscle loss pushed far forward
- Regenerative medicine will eventually provide solutions to wide variety of soft tissue and organ defects, but we are not there yet and we need to stop allowing the media to report that we are
- Gaps have been created in Regen Med product development. Difficult to fund the necessary testing of incremental solutions because the public thinks we've already solved these issues

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- Hanna Baker PhD - Graduate Student, UVA
- Juliana Passipieri PhD - Post-Doctoral Fellow, UVA
- Alexis Gabard – Governmental Affairs, KeraNetics
- Sarah Dyer – Graduate Student, UVA
- Ellen Mintz – Graduate Student, UVA
- Jack Dienes – Graduate Student, UVA

KeraNetics

Questions

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Regen Med Manufacturing and Regulatory Challenges - - > 3 Different Systems

Device Drug and Biologic GMP Requirements		
Medical Devices	Finished Pharmaceuticals	Biologics
Quality System Requirements	Organization and Personnel	Organization and Personnel
Design Controls	Buildings and Facilities	Buildings and Facilities
Document Controls	Equipment	Equipment
Purchasing Controls	Component and Drug Controls	Raw/Starting Material Controls
Identification and Traceability	Product Containers and Closures	Process is the Product
Production and Process Controls	Production and Process Controls	Equipment Controls
Acceptance Activities	Packaging and Labeling Control	Product Stability
Nonconforming Product	Holding and Distribution	Potency and Purity
Labeling/Packaging Controls	Laboratory Controls	Packaging Controls

Regulatory Paths and Compliance		
Medical Devices	Drugs	Biologics
510(k), PMA	IND, NDA	BLA, HCT/P
21 CFR 820, 812, ISO 13485	21 CFR 25, 50, 211, 312, 201-2, 207, 314	21 CFR 25, 207, 211, 600, 361g