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Form Approved
OMB No. 0704-0188

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1. REPORT DATE (DD-MM-YYYY) 11/10/2018	2. REPORT TYPE Presentation	3. DATES COVERED (From - To) 11/10/2018
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4. TITLE AND SUBTITLE Novel Delivery System of Autologous Mesenchymal Stem Cells (MSC) utilizing fabricated scaffold for Bone Regeneration of Compromised Wounds in a Swine Model (Sus scrofa).	5a. CONTRACT NUMBER
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S) Hommas, Zachary	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) 59th Clinical Investigations and Research Support 1100 Wilford Hall Loop, Bldg 4430 JBSA – Lackland, TX 78236-9908 210-292-7141	8. PERFORMING ORGANIZATION REPORT NUMBER 18021
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9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) 59th Clinical Investigations and Research Support 1100 Wilford Hall Loop, Bldg 4430 JBSA – Lackland, TX 78236-9908 210-292-7141	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
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13. SUPPLEMENTARY NOTES
UT research symposium, TX, 11 October 2018

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON SSgt Erin Toth
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (Include area code) 210-292-7141

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59th Medical Wing



Novel Delivery System of Autologous Mesenchymal Stem Cells (MSC) utilizing fabricated scaffold for Bone Regeneration of Compromised Wounds in a Swine Model (*Sus scrofa*)

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The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of Defense, Department of the Army, Department of the Air Force or its Components.

The experiments reported herein were conducted according to the principles set forth in the National Institute of Health Publication 80-23, Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966, as amended.



Background



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- Military personnel are substantially burdened with traumatic extremity bone injury
- Traumatic critical bone injuries are often treated with limb amputation or result in significant loss of function
- No ideal therapy is available to regenerate large bone volumes in compromised wounds
- Traumatic wounds are sub-optimal for bone regeneration
- There is a need for preclinical investigation of a new biologic device designed to regenerate large volumes of bone in compromised wounds



Social Implications



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Military Impact

- Congressional estimation \geq 1500 total American soldier traumatic limb amputations in Afghanistan and Iraqi confrontations
- Estimated life time costs of care for amputees $>$ \$600 billion*

Civilian Impact

- Traumatic injury 2nd leading cause for extremity amputation
- Estimated $>$ 700,000/year[†]
- In 2013 yearly hospital charges of \$8.7 billion

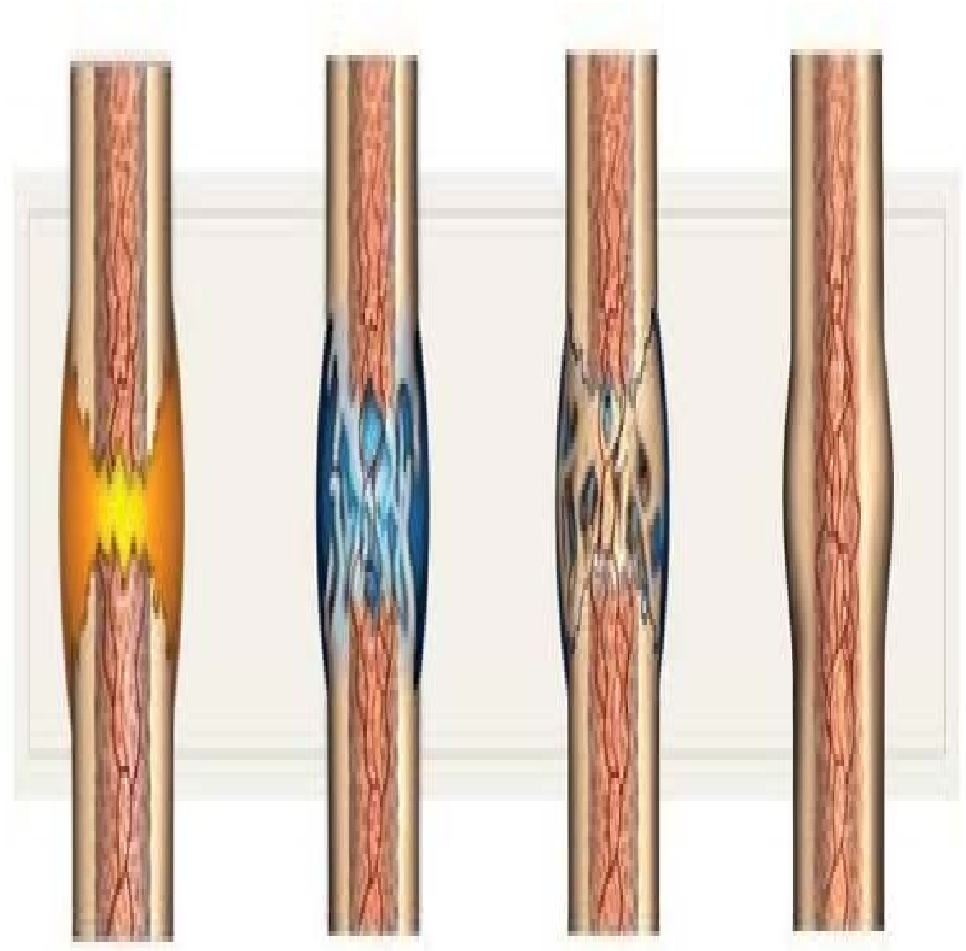


Fracture Healing



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- Injury/Inflammation
- Soft Callus Formation
- Hard Callus formation
- Remodeling



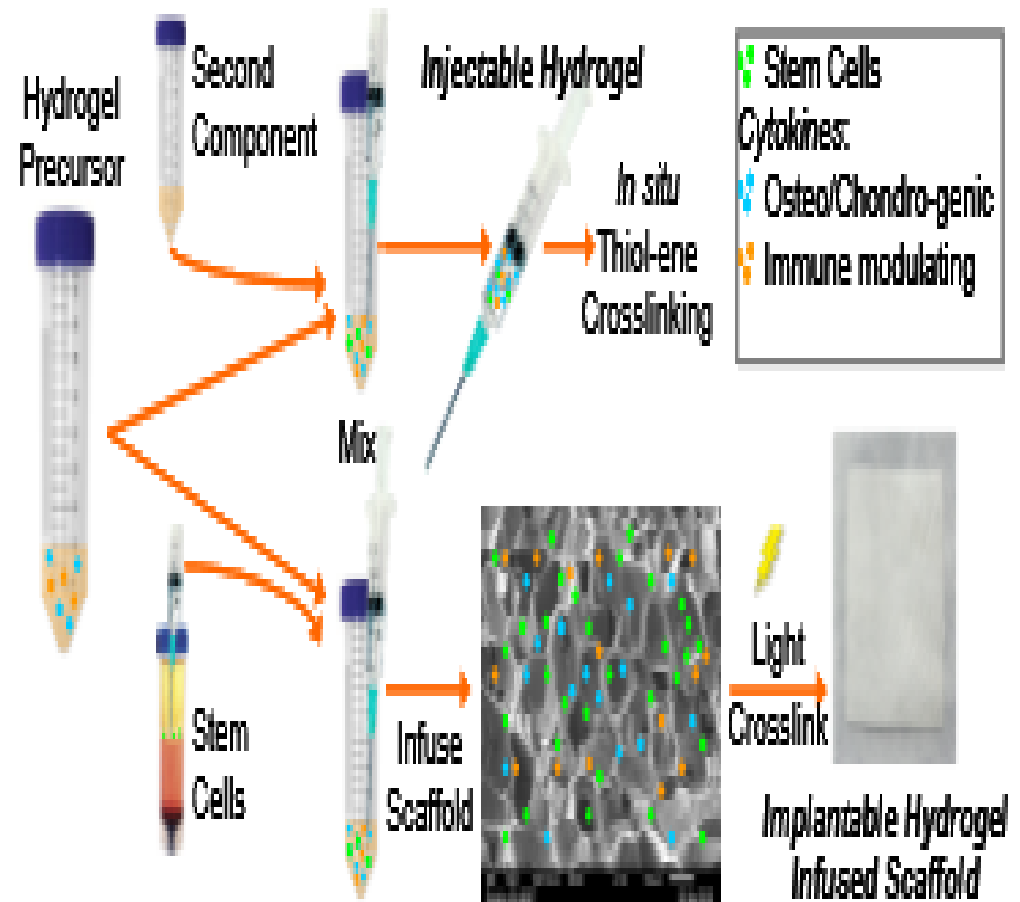


The Biologic Device



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- 1. Hydrogel carrier for cytokines and MSCs
- 2. Nanoparticles for prolonged cytokine delivery
- 3. Chondrogenic and immunomodulatory cytokines
- 4. Porous scaffold providing mechanical support





Hypothesis



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Application of this novel biologic device for the treatment of a comminuted fracture or injuries resulting in a significant bone defect will mitigate hypoxia and non-union.



Objectives



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- Develop a porcine model of comminuted fracture and critical bone defect for the optimization of bone regeneration
- Further the treatment of extremity bone injury
- Determine the immunomodulatory effects of the selected cytokines and MSCs with regards to bone regeneration.



Objectives



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- Cytokines vs. MSCs in local immunomodulation. Are MSCs essential to repair?
- Efficacy results of the coacervate drug delivery
- Skeletogenic results of the hydrogel – possible application as a tissue glue to promote repair of numerous bone injuries
- Bone regeneration results of the integrated scaffold device



Methods



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- Yucatan Swine, Female
- Injectable Device and Implantable device groups
- Survival Endpoints: 1 month and 5 months
- Biosamples/Data Collection
 - Xrays: POD 0, and biweekly until end point
 - Blood sampling for inflammatory markers and Immune cell populations
 - Flow cytometry- Immune cell characterization
 - Cytokine profiling: Identify whether the systemic cytokine response is fibrotic (i.e. Th1 including IFN- γ , TNF- α) versus reparative (e.g. Th2, including IL-10, IL-13).



Methods



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- Immunohistochemistry
 - Lymphocyte subsets
 - M1 and M2 Macrophage Phenotyping
- Histology
 - Safranin O / Fast Green / Hematoxylin staining for cartilage
 - Eosin / Hematoxylin for bone, and Masson's Trichrome for fibrous tissue and the phases of development of bone.
- Transcriptome: comprehensive analysis of growth factor, cytokine, and immune cell gene expression markers using a gene chip.
 - RT-PCR : precisely quantify the expression difference of genes identified in the array.
- Micro-CT of the regenerate tissue for 5 month animals



Experimental Groups



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Injectable Device = 1

Group	Time-points & Replicates			Cipher Name	Scaffold	Hydrogel	IL-10 in coacervate	IL-10 no coacervate	TGFB in coacervate	MSC
	All	1 M = A	5 M = B							
1	9	3	6	Empty						
2	9	3	6	Long		Yes	1 ug/ml		1 ug/ml	
3	9	3	6	Burst		Yes		1 ug/ml	1 ug/ml	
4	9	3	6	ILonly		Yes	1 ug/ml			
5	6	3	3	Control3		Yes				

Implantable Device = 2

Group	Time-points & Replicates			Cipher Name	Scaffold	Hydrogel	IL-10 in coacervate	IL-10 no coacervate	TGFB in coacervate	MSC
	All	1 M = A	5 M = B							
1	9	3	6	Empty						
2	9	3	6	MSC	Yes	Yes	1 ug/ml		1 ug/ml	Yes
3	9	3	6	Long	Yes		1 ug/ml		1 ug/ml	
4	9	3	6	Burst	Yes			1 ug/ml	1 ug/ml	
5	5		5	Golden	Yes	Yes	1 ug/ml		10 ug/ml	Yes
6	9	3	6	Infuse						
7	6	3	3	Control1	Yes					
8	4		4	Control2	Yes	Yes				



Hydrogel



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- Carrier for the cytokines and MSCs and controls the MSC differentiation.
- Preserve and promote cytokine activity via the hydrated environment and direct binding of the cytokines.
- Promotes MSC chondrogenesis and endochondral ossification through
 - Hydrophilic nature
 - Support of spherical cell shape
 - Enhancement of the chondrocyte phenotype.



Nanoparticles



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- Coacervates (aggregates of colloidal droplets) of heparin and a synthetic polymer
- Bind the cytokines via charge interactions
- Stabilize and protect the cytokines and prolong their release.



Cytokines



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- Transforming growth factor beta-3 (TGF β -3) and interleukin-10 (IL-10).
- TGF β -3 is a potent inducer of chondrogenesis by MSCs.
- Both IL-10 and TGF β -3 serve to modulate the immune response
 - Promotes alternate activation of macrophages (M2 phenotype) and a more non-fibrotic healing response



Scaffold



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- Porous scaffold is made from the same polymers as the hydrogel
- Provides structural integrity to the implantable device in large defects.

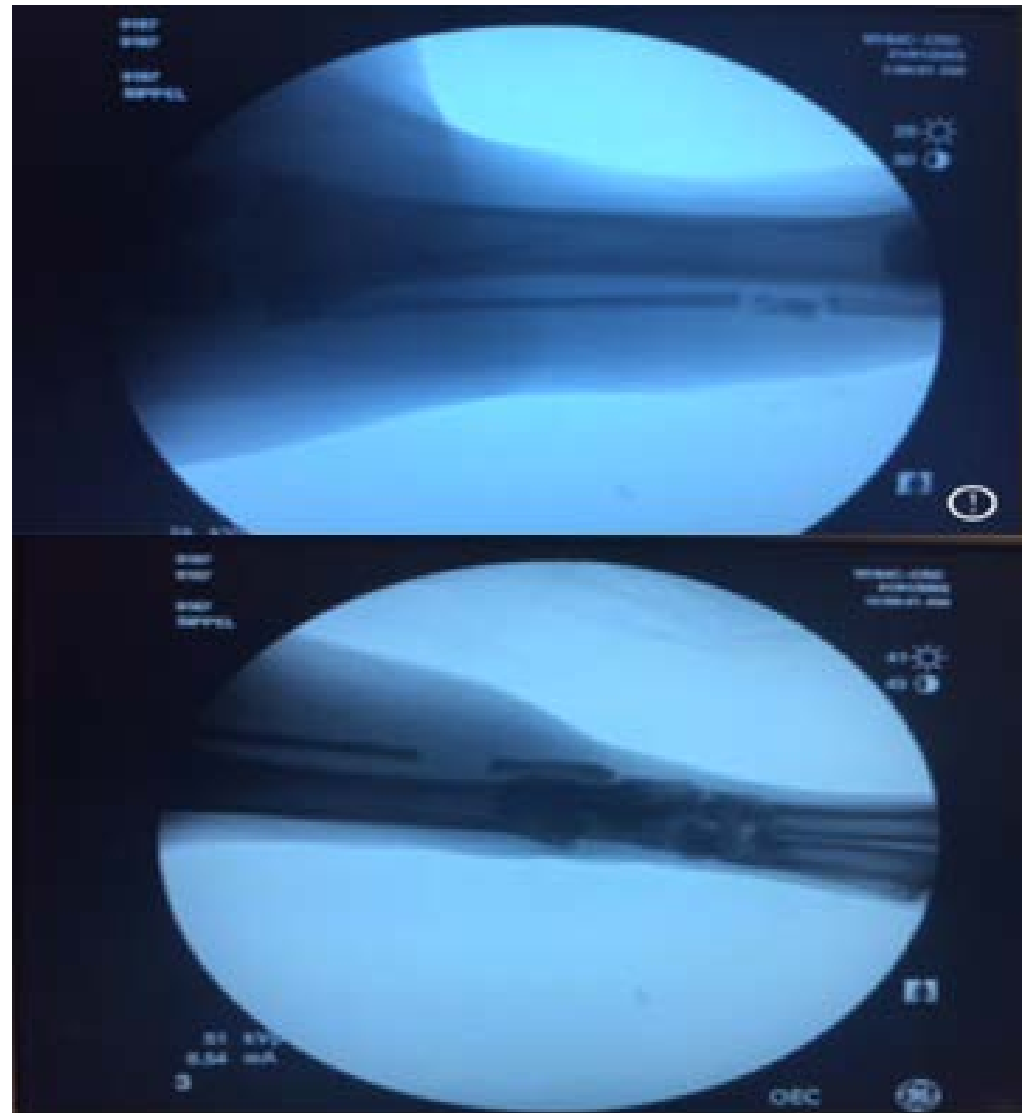


Experimental Model



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- 2 bone injury models in Yucatan swine
 - Comminuted fracture model
 - Critical bone defect models





Scaffold Placement



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Technology Overview



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- MSCs and immunomodulatory cytokines make the wound environment more conducive to regeneration while the hydrogel creates an anti-fibrotic barrier.
- The hydrogel and a chondrogenic cytokine drive cartilage formation by the MSCs.
- Neocartilage is similar to the cartilage callus formed during limb fracture healing and undergoes endochondral ossification to produce bone in wounds with compromised vascularity.



How will this technology be used?



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- **Injectable device** – forward surgical care (percutaneous injection)
 - stabilize bone fragments in comminuted fractures
 - prevent fibrous tissue ingrowth
 - rapidly initiate regeneration.
- **Implantable device** - used in the operating theater
 - promote bone formation
 - minimize non-union when conventional autografts and bone fillers are contraindicated



Summary



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- There is a clear need for technology capable of bone regeneration on critical bone defects in traumatic wounds
- This technology has the potential to mitigate inflammatory processes driving fibrosis and inadequate bone healing
- Injectable device has the potential application for point of care treatment
- Potential to provide reconstructive options when autologous bone is not available
- Broad implications across entire reconstructive and orthopedic surgery fields



Thank You



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