AWARD NUMBER: W81XWH-13-1-0324

TITLE: Improved Healing of Large, Osseous, Segmental Defects by Reverse Dynamization: Evaluation in a Sheep Model

PRINCIPAL INVESTIGATOR: Christopher H. Evans

CONTRACTING ORGANIZATION: Mayo Clinic
Rochester, MN 55905

REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE			*Form Approved* *OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2015	Annual	09/30/2014 – 09/29/2015

4. TITLE AND SUBTITLE

Improved Healing of Large, Osseous, Segmental Defects by Reverse Dynamization: Evaluation in a Sheep Model

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-1-0324

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Christopher H. Evans, Ph.D.
Nicholas P. Quirk

E-Mail: evans.christopher@mayo.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Mayo Clinic
200 First Street, SW
Rochester, MN 55905

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Mechanical testing of the finalized fixator design was completed during course of current project year. Using cadaveric sheep tibiae it was found that when the fixator was modulated from low to high stiffness, the stiffness doubled, which is sufficiently close to desired increment of 2.5-fold to justify the in vivo studies on living sheep. To confirm that the fixator could bear the forces imposed by adult, ambulating sheep, a plastic yield point above 500N was determined. Moreover, negligible inter-fragmentary movement differences of the fracture gap occurred during repeated load-cycling to mimic the projected lifecycle of the fixator while attached to the sheep. This information has been coupled with a finite element analysis model of the mechanical stress properties of the fixator. This completes SOW tasks 1-3. Finalization of surgical procedure (through mock surgeries) and animal husbandry protocols have been completed. This will allow a seamless transition into the live animal surgeries (SOW task 4).

15. SUBJECT TERMS
Sheep; tibia; external fixator; dynamization, mechanical characterization

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	10	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

1. Introduction

Large segmental defects in long bones do not heal well and represent a major clinical problem [1]. INFUSE®, comprising recombinant human bone morphogenetic protein-2 (rhBMP-2) delivered on an absorbable collagen sponge, is used by surgeons to assist the healing of large osseous lesions but the clinical results have been disappointing [2]. Moreover, INFUSE® is very expensive.

It is well established that bone healing is influenced by the mechanical environment [3] [4]. Segmental defects may be stabilized mechanically by an external fixator. There has been much interest in the concept of dynamization, whereby the defect is first stabilized rigidly to initiate healing and then subjected to axial motion (dynamization) to promote the subsequent stages of healing and maturation [5]. This axial motion is transmitted as an axial strain or interfragmentary movement (IFM) through the separated bone cortices (fracture gap).

In research funded by a CDMRP Idea Development Award, we used a rat segmental defect model to show that healing in response to rhBMP-2 could be accelerated and improved by “reverse dynamization” in which the fixator is first applied in a loose configuration and then stiffened once bone formation had started [3], [6].

The present research will determine whether reverse dynamization is also effective in sheep, as a stepping stone towards human, clinical trials.

2. Keywords

Bone healing; segmental defect; reverse dynamization; sheep; external fixator

3. Overall Project Summary

The period covered by this annual report was dedicated to mechanical characterization and design refinement of the external fixator (SOW tasks 1-3). Finalizing pre-operative planning for the live animal study (SOW task 4) was also completed. Throughout the stages of mechanical testing, the fixator was constantly assessed for improvements in all areas. Broadly, refinements to material finish (for higher fixator fatigue properties) addition of sand blasting to manufacturing process (for greater pin-to-fixator friction force), improved pin placement (due to pin engagement issues) and multiple shim design modifications (for ease of use and greater surface area for dynamizing shims) were made. In addition multiple revisions were made to surgical approach and tools utilized for reproducible, safe, efficient surgeries.

Thus SOW tasks 1-3 have been completed, with the successful property analysis and mechanical characterization of the prototype external fixator in the ovine critical size defect animal model. This work allows us to start SOW task 4 (live animal study) of this study with all required knowledge of the fixator assemblies mechanical properties, its final optimized design and animal handling care and surgical details determined.

4. *Key Research Accomplishments*

I. External fixator mechanically characterized

Initial fixator efficacy was validated with sawbones, a non-biological, repeatable, testing material representative of cortical bone and widely accepted in the literature (Figure 1) [7]. Following testing with sawbones, further tests on cadaveric sheep tibiae were conducted to confirm the sawbone testing results. Measurement of axial stiffness and inter-fragmentary motion (IFM) was the main focus of the mechanical testing. Testing was taken to a maximum of 500N axial compression, which was determined as the greatest possible force generated during the full ovine gait cycle [8].

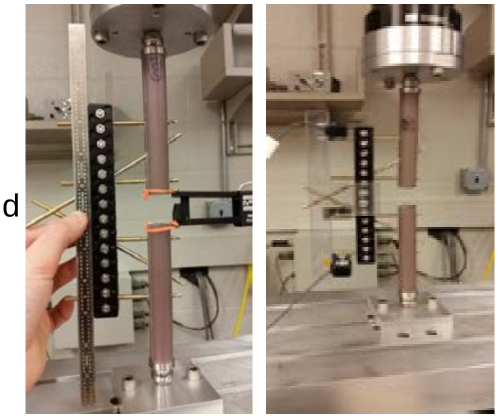


Figure 1 Sawbone models used for repeatable external fixator testing and testing fixtures for attachment to MTMS machine. Also shown are extensometers in use, one across fracture gap to measure IFM (Left) and two used for fixator deformation assessment attached to custom rig (Right).

This showed that within 500N axial loading, the fixator remains within the elastic deformation region; (i.e. the fixator is not being permanently deformed) (Figure 2). Figure 2 also shows slight yield at ~200N force, which was rectified by pin placement adjustments. Axial stiffness across sawbone or bone samples approximately doubled when shims were inserted: high stiffness configuration (shims in) - 562 N/mm; low stiffness configuration (shims out) - 293 N/mm (Figure 2).

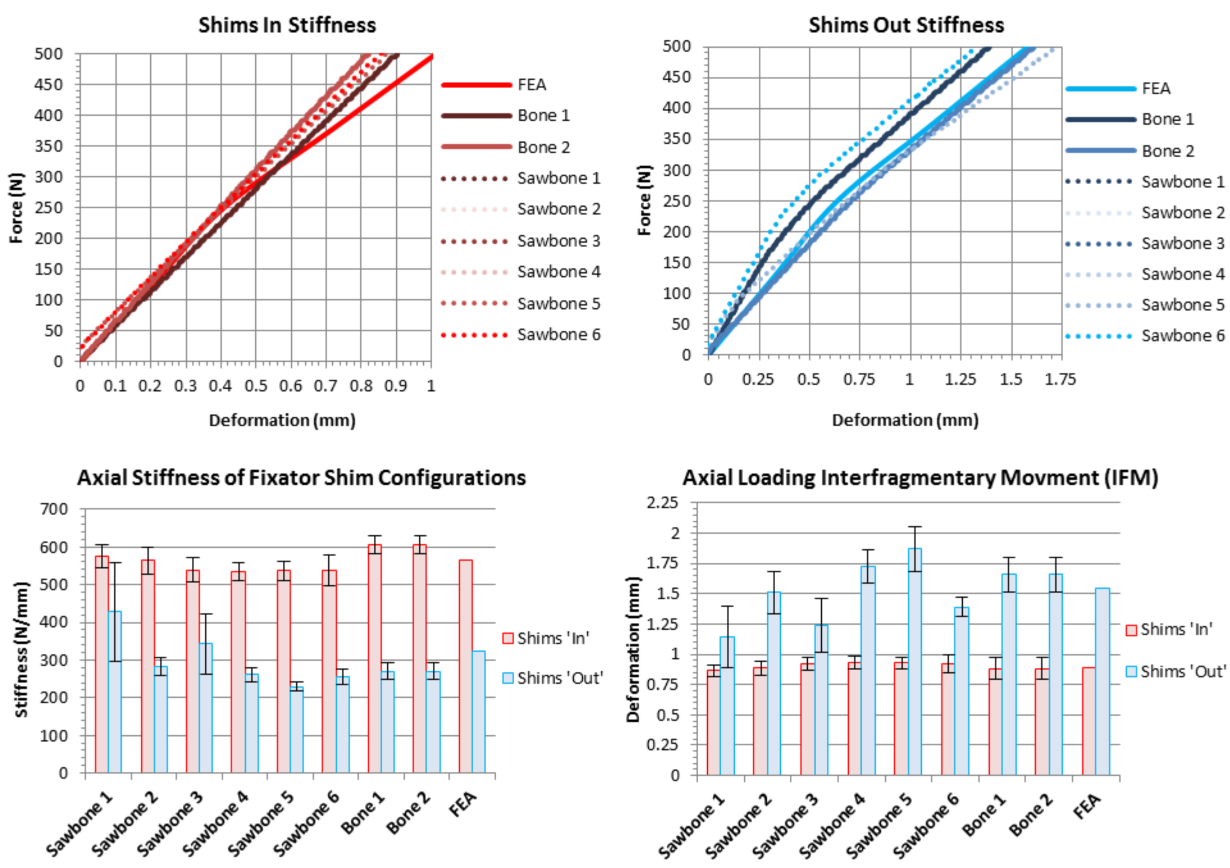


Figure 2 Top: Elastic axial compression testing stiffness plots of external fixators, shims in ***(left)*** and shims out ***(right)*** configurations shown. **Bottom:** Comparison plots of sample axial stiffness ***(left)*** and interfragmentary movement ***(right)***

Fixator deformation assessments were also conducted to determine loading parameters during axial compression. The fixator was confirmed to deform over the 'neutral axis' (center of fixator) with negligible plastic deformation within a 500N force range during cyclic testing (Figure 3). Custom rigs incorporating 2 extensometers were attached to the neutral axis of the fixator (Figures 1 and 3) and 'bending' was determined during axial elastic loading tests. The data confirm that the fixator deforms symmetrically over the neutral axis, confirming the manufacturing design purpose of uniform IFM, with negligible subsidence. Due to material differences between the fixator (aluminum) and pins (titanium), coupled with fatigue mechanics, slight plastic deformation was expected with the 500N 'elastic' axial compression force range.

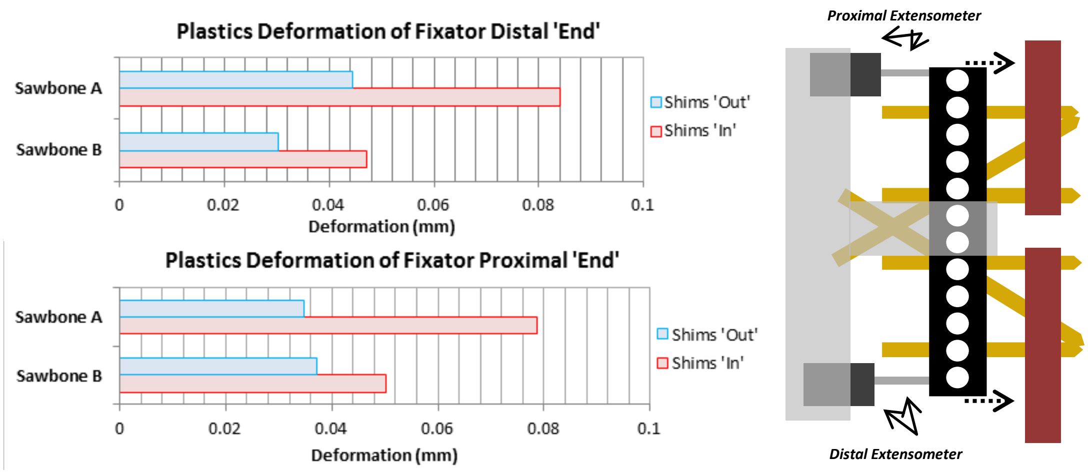


Figure 3 Left: Plastic deformation of fixator's across cyclic 500N axial load, Proximal ***(top)*** and Distal ***(bottom)***. **Right:** Schematic of fixator body deformation with labelled proximal / distal extensometers and fixator 'end' deformation direction.

Cyclic fatigue testing was conducted over two experimental (dynamized / reverse-dynamized) animal group models. Testing at 4Hz, 500N maximum force, 100,000 cycles represented worst case animal loading for initial 3 month testing period (Figure 4). Significantly, following initial stabilization period in all configurations (due to testing fixture/fixator subsidence), all deformation plateaued. Coupled with physical fixator assessments following testing for damage, this shows efficacy of fixator assembly in animal model.

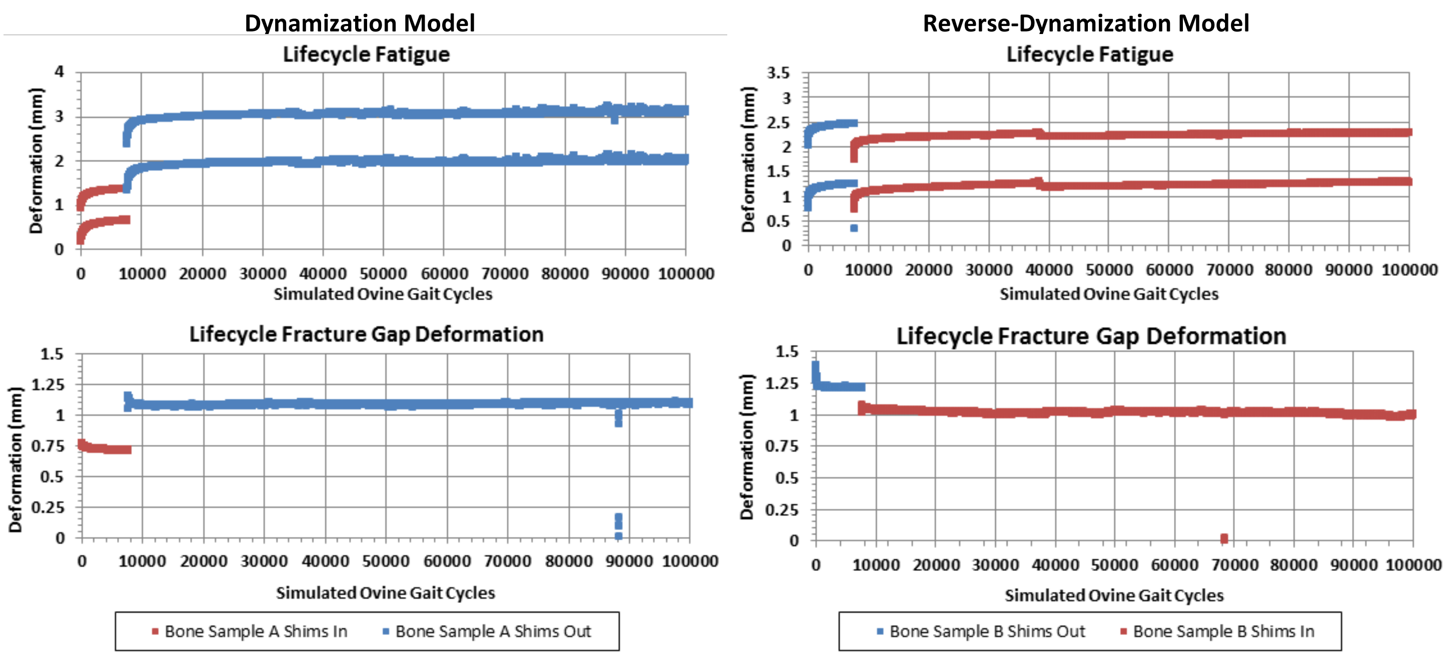


Figure 4 Life cycle axial cyclic testing (3 month period). **Top:** Min/Max fracture gap deformation measured. **Bottom:** Calculated fracture gap deformation occurring throughout testing. Note: Fracture gap deformation does not represent complete IFM as testing profile did not consistently return to 0N at each cycle start.

Mechanical testing also assessed plastic deformation and failure point of fixators (Figure 5). Ultimate tensile strength and failure were not reached. However the yield points for the shims out configuration (~ 510N) and shims in configuration (~ 550N) were determined. These values corroborate those from previous cadaveric testing. This shows that any lesser force will only negligibly deform the fixator.

Final mechanical testing assessed the torsional stiffness of the fixator. Figure 5 also shows torsional stiffness for shims in vs shims out configurations. The shims in configuration resulted in lower torsional stiffness due to the pin configuration localizing torsional stress concentration in the fixator. Overall the torsional stiffness is sufficient to support the ovine animal model.

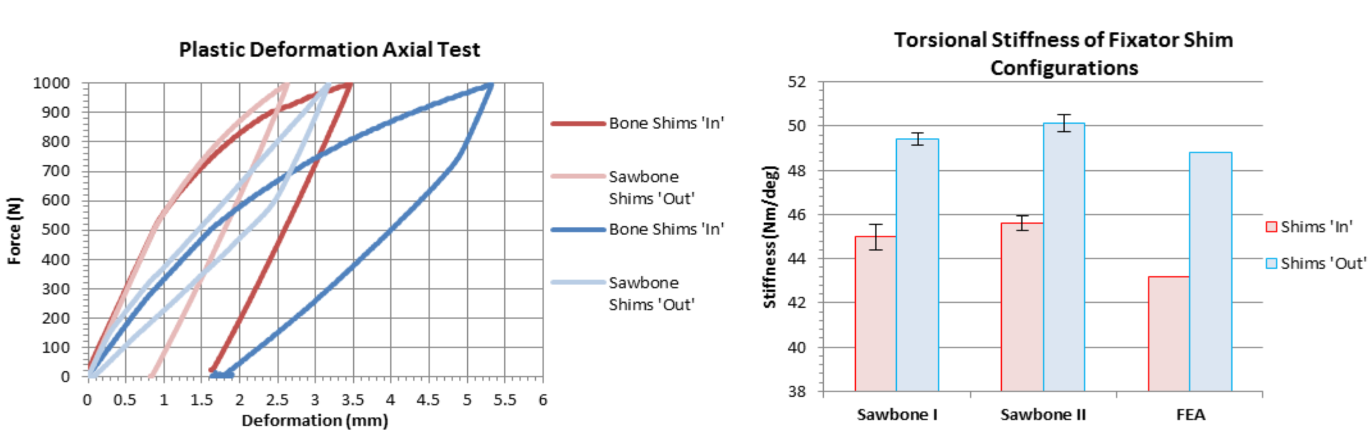


Figure 5 Axial Plastic Deformation Compressive testing (Left) and Elastic Torsional Stiffness of Fixator (Right)

II. Finite Element Analysis modelling

A Finite Element Analysis (FEA) model was established and refined. Although refinement is continuing, the model accurately predicts the mechanical environment of the fixator assembly and further validates mechanical testing. Conversely, mechanical testing validates the FEA model and thus can be used for greater investigation into the stress mechanics of the fixator through differing loading parameters. The FEA model has successfully evaluated axial elastic loading results (Figure 2) and elastic torsional results (Figure 5). The model incorporates 624,494 nodes and 390,052 elements (tetrahedrons) (Figure 6). The stresses shown mainly occur at pin – fixator /pin – sawbone junction (as expected) and throughout the center span of the fixator.

Shims ‘In’ **Shims ‘Out’ Model**

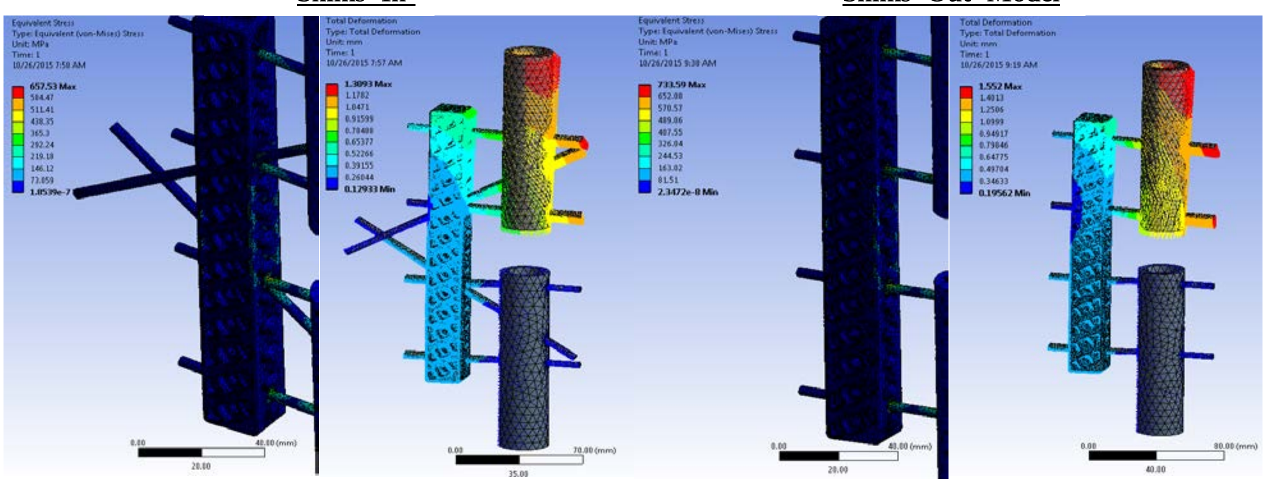


Figure 6 Finite Element Analysis (FEA) model developed through ANSYS 16. Stresses and total deformation suffered shown in shims in configuration ***(left)*** and shims out configuration ***(right)***. Shims out model simplified by removal of ‘diagonal’ pins for ease of computation (not in contact with fixator without shim addition).

5. Conclusion

Successful mechanical characterization of the external fixator has been accomplished, which allowed for final iteration of fixator and associated components. Coupled with all live animal study training /and administration requirements completed, we are now in position to begin the next stage of the project.

6. Publications, Abstracts and Presentations

None

7. Inventions, Patents and Licenses

None

8. Reportable Outcomes

- Prototype adjustable stiffness external fixator suitably mechanically characterized
- External fixator animal model efficacy confirmed
- All pre-operative planning required for live animal study completed

9. Other Achievements

None

References

[1] A. N. Pollak and J. R. Ficke, "Extremity war injuries: challenges in definitive reconstruction.," *J. Am. Acad. Orthop. Surg.*, vol. 16, no. 11, pp. 628–634, 2008.

[2] E. J. Carragee, E. L. Hurwitz, and B. K. Weiner, "A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned.," *Spine J.*, vol. 11, no. 6, pp. 471–491, 2011.

[3] V. Glatt, M. Miller, a Ivkovic, F. Liu, N. Parry, D. Griffin, M. Vrahas, and C. Evans, "Improved healing of large segmental defects in the rat femur by reverse dynamization in the presence of bone morphogenetic protein-2," *J. bone Jt. Surg. Am. Vol.*, vol. 94, no. 22, pp. 2063–2073, 2012.

[4] M. Mehta, S. Checa, J. Lienau, D. Hutmacher, and G. N. Duda, "In vivo tracking of segmental bone defect healing reveals that callus patterning is related to early mechanical stimuli.," *Eur. Cell. Mater.*, vol. 24, no. 0, pp. 358–71; discussion 371, 2012.

[5] G. De Bastiani, R. Aldegheri, and L. Renzi Brivio, "The treatment of fractures with a dynamic axial fixator.," *J. Bone Joint Surg. Br.*, vol. 66, pp. 538–545, 1984.

[6] V. Glatt, C. H. Evans, and R. Matthys, "Design, characterisation and in vivo testing of a new, adjustable stiffness, external fixator for the rat femur.," *Eur. Cell. Mater.*, vol. 23, pp. 289–98; discussion 299, 2012.

[7] C. A. Grant, M. Schuetz, and D. Epari, "Mechanical testing of internal fixation devices: A theoretical and practical examination of current methods," *J. Biomech.*, Oct. 2015.

[8] R. J. Nesbitt, S. T. Herfat, D. V. Boguszewski, A. J. Engel, M. T. Galloway, and J. T. Shearn, "Primary and secondary restraints of human and ovine knees for simulated in vivo gait kinematics," *J. Biomech.*, vol. 47, no. 9, pp. 2022–2027, 2014.

Appendix

a) Quad Chart

Improved Healing of Large, Osseous, Segmental Defects by Reverse Dynamization: Evaluation in a Sheep Model

Log number OR120192 / Quarterly Technical Progress Report

Award Number W81XWH-13-1-0324

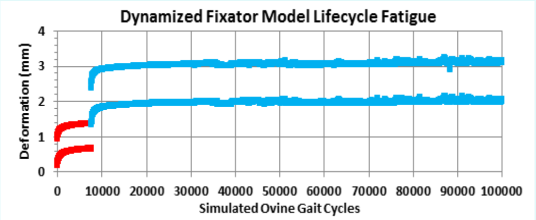
PI: Evans, Christopher **Org:** Mayo Clinic **Award Amount:** $825,769

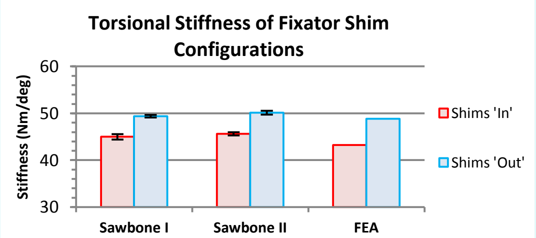
Study/Product Aim(s)

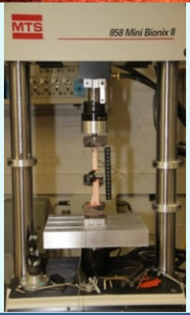
- To design, construct, characterize and evaluate a scalable, adjustable stiffness, external fixator that is appropriate for use in sheep and will allow reverse dynamization in a clinically expeditious manner.
- To evaluate the ability of reverse dynamization to enhance healing of a 3 cm, tibial defect in sheep.

Approach

We will first design and construct an external fixator that can be applied to a fractured sheep tibia, allowing us to alter the stiffness of fixation while it is attached to the bone. The mechanical properties of the fixator will be evaluated and characterized. The final design will be used in a sheep, tibial segmental defect model. Fixation of the defect will be initially loose. Once bone has started to form, stiffness will be increased and healing monitored.







Accomplishment: SOW 1-4 completed(final testing of cyclic axial bone fracture gap and torsional testing shown). *In vivo* sheep studies will now be commenced.

Timeline and Cost

Activities CY	13	14	15	16
Fixator design, characterization				
Initiate in vivo studies on sheep				
Complete in vivo studies				
Text (Major aim/study/milestone)				
Estimated Budget ($K)	**$219,808**	**$334,274**	**$301,876**	

Updated: Mayo Clinic 10/2015

Goals/Milestones

CY13 Goal – Finish fixator design; construction and evaluation of prototype.

CY14 Goals – Initiate in vivo studies in which fixators will be used to accelerate the healing of 3cm defects in sheep tibiae.

CY15 Goals – Completion of sheep studies. Preparation of manuscripts for publication.

Comments/Challenges/Issues/Concerns

- Slight delay due to differing surgical approach investigation, no concern.
- Further modifications to fixator component design; shims changed to allow greater ease of use and surface area (higher pin-fixator contact and friction force).

Budget Expenditure to Date:

Projected Expenditure: $580,516

Actual Expenditure: $464,290