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# Acceleration of Fracture Healing: a Comparison Between Clinical Available Low Intensity Pulsed Ultrasound (LIPUS) and a Novel BiModal Acoustic Signal System

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**Abstract**—***Ultrasound (US) accelerates healing by stimulating the production of bone callus and the process of mineralization. A US system (EXOGEN; Bioventus, Durham, NC, USA) using low intensity pulsed ultrasound (LIPUS) and this principle is FDA-approved. This study analyzed the effectiveness of a novel bimodal acoustic signal (BMAS) for bone fracture healing compared to the clinically used LIPUS system in an animal model. Seventeen mature white New Zealand female rabbits, underwent a bilateral fibula osteotomy as part of an IACUC-approved protocol. Afterwards, each rabbits' legs were randomized to receive 20 minutes treatment daily for 18 days with BMAS or EXOGEN. The latter utilizes a longitudinal ultrasonic mode only, while the former employs US-induced shear stress to promote bone formation. Power Doppler imaging (PDI) was acquired days 0, 2, 4, 7, 11, 14 and 18 post-surgery to monitor treatment response and local inflammation. Images were analyzed off-line using ImageJ (NIH, Bethesda, MD, USA) for amount and intensity of flow. X-rays were acquired to evaluate fractures on days 0, 14, 18 and 21 post-surgery. Rabbits were euthanized day 21 post-surgery, the legs were extracted and their fibulas were analyzed with an electromechanical device to determine maximum torque, initial torsional stiffness and angular displacement at failure. The legs also underwent CT DEXA analysis to determine the ratio of bone mineral density (BMD) or bone mineral content (BMC) values at the fracture level over normal bone values. ANOVAs and paired t-tests were used to compare pair-wise outcome variables for the 2 treatment modes on a per rabbit basis. The BMAS system induced better fracture healing with greater stiffness (0.042 ± 0.099 Ncm/deg, p = 0.050) and torque (1.574 ± 2.953 Ncm, p = 0.022) at the fracture sites than the LIPUS (EXOGEN) system. Quantitative PDI assessments showed a higher relative amount of vascularity (i.e., difference in pixel counts) with LIPUS (EXOGEN) than BMAS on days 4 and 18 (p < 0.04). The BMD and BMC analysis showed no significant statistical difference between BMAS and LIPUS (EXOGEN); p > 0.25. The novel BMAS technique achieved better bone fracture healing response than the current FDA-approved LIPUS (EXOGEN) system.***

***Keywords—ultrasound, LIPUS, bone fracture healing, animal model***

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## I. INTRODUCTION

The biological process for fracture healing consists of three distinct phases: an inflammatory phase with cell proliferation, a chondrogenic phase with cartilage hypertrophy and an angiogenesis/osteogenic phase with woven bone cartilage replacement and remodeling [1]. The process of bone fracture healing fails in around 5 to 10% of patients [1-6]. Several methods to enhance fracture healing were studied over the past 50 years, among them mechanical stimulation, electromagnetic fields and low-intensity pulsed ultrasound (LIPUS) [2, 7, 8]. LIPUS uses acoustic pressure waves transmitted by ultrasound devices to accelerate bone fracture healing by stimulating the production of bone callus and the process of mineralization. LIPUS utilizes a longitudinal ultrasonic mode that is transmitted transcutaneously by high-frequency acoustic pressure waves, with an intensity (30 mW/cm$^2$) in the low end of the range used for diagnostic purposes (up to 720 mW/cm$^2$) that is regarded as non-thermal and non-destructive [2, 7]. A system using this principle (EXOGEN, Bioventus, Durham, NC, USA) is FDA-approved and it has been clinically used for over 15 years to accelerate bone healing, with a 24-38% reduction in bone fracture healing time [1-3, 8-11]. Specifically, the EXOGEN system utilizes 1.5 MHz sinusoidal waves modulated in bursts of 200 ms at a repetition frequency of 1 kHz, and a spatial average–temporal average intensity of 30 mW/cm$^2$ [2, 3, 9].

The novel bimodal acoustic signal (BMAS) system employs ultrasound-induced shear stress to promote bone formation. The propagating resultant longitudinal acoustic signal:

- Reflects longitudinal signal where the reflection angle equals the incident angle.
- Bimodal signal is transmitted into the interior of the bone, propagating as both shear and longitudinal waves.

The objective of the study was to analyze the effectiveness of this novel bimodal acoustic signal (BMAS) for bone fracture healing compared to the current FDA-approved LIPUS device (EXOGEN) in an animal model.

## II. MATERIAL AND METHODS

### A. Animal Model

The animal model chosen for this study was based on prior studies that confirmed the validity of low intensity pulsed ultrasound (LIPUS), which is the device currently clinically used to decrease bone healing time. Also, it served as the control for this study.

The animal model consisted of white New Zealand female mature rabbits, the gender and age group of the rabbits was kept consisted to diminish biological variability. This study was conducted from April 2017 to June 2018 at Thomas Jefferson University (Philadelphia, PA, USA) with Institutional Animal Care and Use Committee (IACUC) approval. A total of 17 rabbits were used during the study, with an average weight of 3.3 kg, divided in 5 cohorts of 3 or 4 rabbits each.

Each cohort went through a 22 days study, with day 0 being the surgical day, days 1-18 as the treatment period, days 19-20 the rest period (not treatment days) and finally day 21 was the euthanasia day to finalize the study (Figure 1).

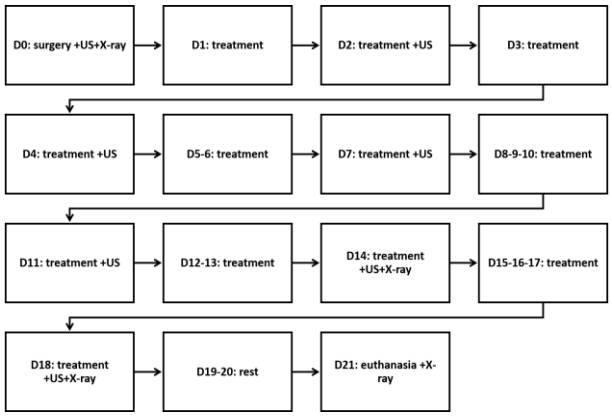


Figure 1: Study timeline for each cohort.

### B. Surgical procedure

The rabbits underwent a surgical procedure under general anesthesia consisting of a bilateral fibula osteotomy, where a rotary tool (Figure 2) was used to cause a clean transverse cut in the 1/3 proximal length of the fibula. After the surgical procedure X-ray images were acquired to show the exact location and extension of the fracture (Figure 3a). Moreover, an ultrasound examination was performed using B-mode imaging to measure the distance from the fracture site to the bone extremities the fracture and power Doppler was acquired as a biomarker for the inflammation process during healing (Figures 3b and 3c). A permanent marker was used to mark the skin surface directly over the fracture site to keep the probe location exactly where it was needed to be during the duration of the study.


Figure 2: Rotary tool used during surgical procedure.

### C. Treatment

The rabbits underwent 18 consecutive days of treatment, where each rabbit had their legs randomized to receive 20 minutes treatment daily with BMAS or with EXOGEN. That way every rabbit received both the treatment and the control device to limit biological variability.

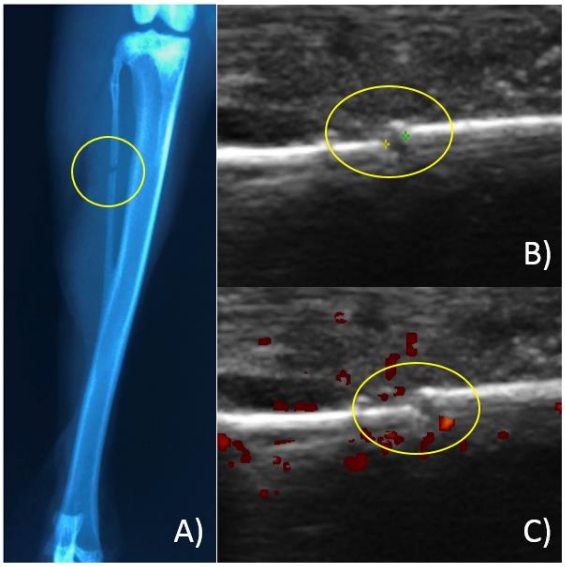


Figure 3: Imaging techniques post-surgical procedure showing the fracture site inside the yellow circle. A: X-ray. B: B-mode ultrasound. C: Power Doppler ultrasound.

### D. Imaging

X-ray images were acquired to evaluate fractures on days 0, 14, 18 and 21 post-surgery to monitor bone callus formation, using a Summit X-ray (InnoVet, Niles, IL, USA). Power Doppler imaging (PDI) were acquired days 0, 2, 4, 7, 11, 14 and 18 post-surgery to monitor local inflammation, using an S9 scanner (SonoScape, Shenzhen, China) with a linear probe (L742). PDI images were analyzed off-line using ImageJ (NIH, Bethesda, MD, USA) for the amount and intensity of vascularity at the fracture site.

### E. End of study

Rabbits were euthanized day 21 post-surgery. The legs were extracted and their fibulas were analyzed with an electromechanical device to determine maximum torque, initial torsional stiffness and angular displacement at failure.

The legs also underwent μCT DEXA analysis to determine the ratio of bone mineral density (BMD) and bone mineral content (BMC) values at the fracture site over normal bone values acquired proximal and distal to the fracture site.

*F. Statistical Analysis:*

ANOVAs were used to compare pair-wise outcome variables on a per rabbit basis. All tests were performed using Stata 15.1 (Stata Corp, College Station, TX, USA).

## III. RESULTS

All data analysis were done using a pair-wise outcome variables comparison on a per rabbit basis to avoid biological variability.

The PDI and X-ray images qualitative assessments showed no statistical differences at any time-point between LIPUS (EXOGEN) and BMAS ($p > 0.25$).

The PDI quantitative assessments where the amount and intensity of vascularity at the fracture site were analyzed showed a higher pixel count with LIPUS (EXOGEN) than BMAS on days 4 and 18:

- D4: -492 ± 734, p = 0.007
- D18: -284 ± 613, p = 0.037

For the remaining time points (D0, D2, D7, D10 and D14), the PDI quantitative assessments showed no significant statistical difference between LIPUS (EXOGEN) and BMAS ($p > 0.25$).

The analysis using an electromechanical device to determine maximum torque, initial torsional stiffness and angular displacement at failure showed that BMAS induced better fracture healing with greater stiffness and torque at the fracture sites than LIPUS (EXOGEN) (Figure 4):

- Stiffness: 0.042 ± 0.099 Ncm/deg, p = 0.050.
- Torque: 1.574 ± 2.953 Ncm, p = 0.022.

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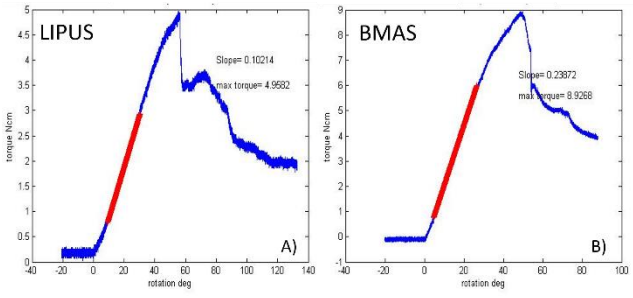


Figure 4: Example of the graphs created by the electromechanical analysis of torque and stiffness. A: LIPUS (EXOGEN). B: BMAS.

The μCT DEXA analysis of the BMD and BMC values obtained at the fracture site and from normal bone tissue areas proximal and distal to the fracture site showed no significant statistical difference between the BMAS and LIPUS (EXOGEN) outcomes (Figure 5):

- BMD ratio average: 0.72 vs. 0.70; $p > 0.25$.
- BMC ratio average: 0.69 vs. 0.68; $p > 0.25$.

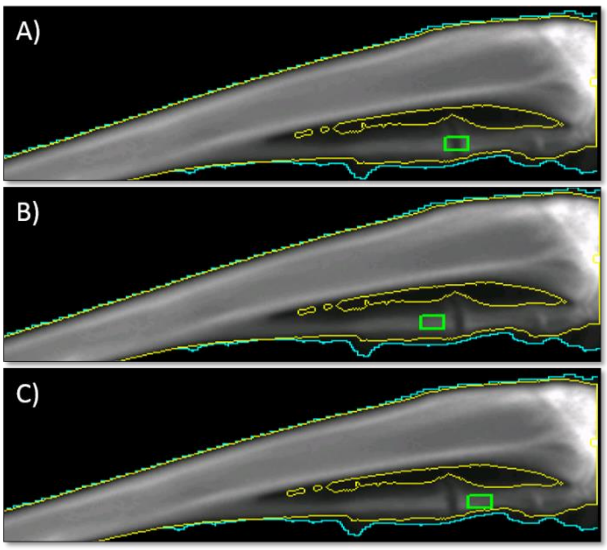


Figure 5: Example of the DEXA analysis where the green rectangle represents the region of interest (ROI) for the data analysis. A: ROI at the fracture site. B: ROI distal to the fracture site. C: ROI proximal to the fracture site.

## IV. DISCUSSION

The use of LIPUS to accelerate the fracture repair process in humans was first reported by Xavier and Duarte in 1983 [13]. Several studies were done after this first report leading to the approval of the EXOGEN system (which is a LIPUS system) by the U.S. Food and Drug Administration (FDA) in 1994 approval of EXOGEN by the FDA, for the accelerated healing of certain fresh fractures [1, 9, 13]. In 2000 the approval was extended for the treatment of established non-union fractures [1, 9. 13].

The clinical use of LIPUS accelerate bone healing has been well established in the literature, with a 24-38% reduction in bone fracture healing time [1-3, 8-11]. LIPUS utilizes a longitudinal ultrasonic mode that is transmitted transcutaneously by high-frequency acoustic pressure waves, with a relatively low intensity (30 mW/cm2) [2, 7].

The novel BMAS system is also a LIPUS system, however the system also employs an ultrasound-induced shear stress to promote bone formation. The propagating resultant longitudinal acoustic signal reflects both a longitudinal signal (where the reflection angle equals the incident angle) and a bimodal signal that is transmitted into the interior of the bone, propagating as both shear and longitudinal waves. The hypothesis of this study was to determine if BMAS with the added components

described above would increase the acceleration of bone fracture healing when compared with the clinically used LIPUS (EXOGEN) system.

The analysis of the bone structure done with the BMC and BMD values acquired with μCT DEXA scans showed no significant statistical difference between BMAS and LIPUS (EXOGEN), with p-values above 0.25 for both BMD and BMC ratio average. More studies are necessary to evaluate how the bone structure responds to both systems.

Rawool et al. described a study in 2003 that evaluated local inflammation at the fracture site in six dogs (3 pairs), where one dog in each pair was treated with LIPUS system, and the other was used as a control [14]. The results showed that in the first 14 days post-fracture there was a blood flow increase in both treatment and control, with the treatment group having 33% more vascularity than the control group.

Our study used PDI at 7 different time points (D0, D2, D4, D7, D11, D14 and D18) to evaluate blood flow at the fracture site for both LIPUS (EXOGEN) and BMAS. Only 2 time points showed a significant statistical difference with LIPUS (EXOGEN) having a higher pixel count (measurement of vascularity) than BMAS (D4 and D18; $p < 0.04$). For the remaining time points (D0, D2, D7, D10 and D14), there was no significant statistical difference ($p > 0.25$). Our first hypothesis was that BMAS would demonstrate a higher degree of vascularity at the fracture site, since it was expected to increase the acceleration of bone fracture healing, however this was not seen. There is a need to study the local inflammation threshold where the benefits of the local inflammation are supersede by a destructive inflammation to be able to better understand the vascularity at the fracture site.

The results of the study support the hypothesis that BMAS decreases bone fracture healing time when compared with the LIPUS (EXOGEN) system with BMAS inducing better fracture healing with greater stiffness and torque at the fracture sites (stiffness: $p = 0.050$ and torque: $p = 0.022$), albeit in a small sample size.

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