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PRINCIPAL INVESTIGATOR: Julianna Simon

CONTRACTING ORGANIZATION: The Pennsylvania State University

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14. ABSTRACT The purpose of the project is to investigate Doppler ultrasound imaging and therapeutic ultrasound histotripsy for the diagnosis and treatment of acquired heterotopic ossification (HO). Thus far, we have found the Doppler ultrasound parameters within FDA limits that produce the highest signal in ossified cells and have used these in a murine model of acquired HO. Preliminary results show we can detect HO in some mice as early as 3 days after induction of HO, with the signal increasing as HO develops. Full results from the animal study are pending receipt of chemicals needed for histological stains. A manuscript of these results in ossified cells and mice is in preparation. We have also shown that therapeutic ultrasound histotripsy at 1.07 MHz with acoustic pressures of 17 MPa positive and 6 MPa negative successfully fractionates ossified cells with minimal damage to the surrounding cells and tissue-mimicking phantom. Murine studies to treat HO are poised to begin as we confirm our results with one final round of ossified stem cells. A manuscript on treating HO in ossified cells and mice will be prepared once all studies have been completed.					
15. SUBJECT TERMS Heterotopic ossification, Doppler ultrasound, twinkling artifact, therapeutic ultrasound, histotripsy, mineralization					
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TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	7
5. Changes/Problems	8
6. Products	9
7. Participants & Other Collaborating Organizations	10
8. Special Reporting Requirements	12
9. Appendices	12

1. INTRODUCTION:

Acquired heterotopic ossification (HO), or the presence of bone in soft tissues where bone does not normally exist, is the most common form of HO and occurs in <64% of military blast injuries to an extremity. Yet HO cannot be detected by computed tomography until at least 2 months post injury and physicians wait more than a year for HO to mature before treating by surgical resection to reduce the risk of recurrence. In this project, we are (1) evaluating the color Doppler ultrasound twinkling artifact to detect early signs of HO in mineral-depositing cells and in a murine model of HO; and (2) evaluating ultrasound-guided therapeutic ultrasound histotripsy to treat early HO in mineralized cells embedded in a tissue-mimicking phantom and in a murine model of HO. The goal of this project is to provide proof-of-concept for the detection and disruption of HO with ultrasound.

2. KEYWORDS:

Heterotopic ossification, Doppler ultrasound, twinkling artifact, therapeutic ultrasound, histotripsy, mineralization, human bone-marrow derived stem cells, osteogenic media

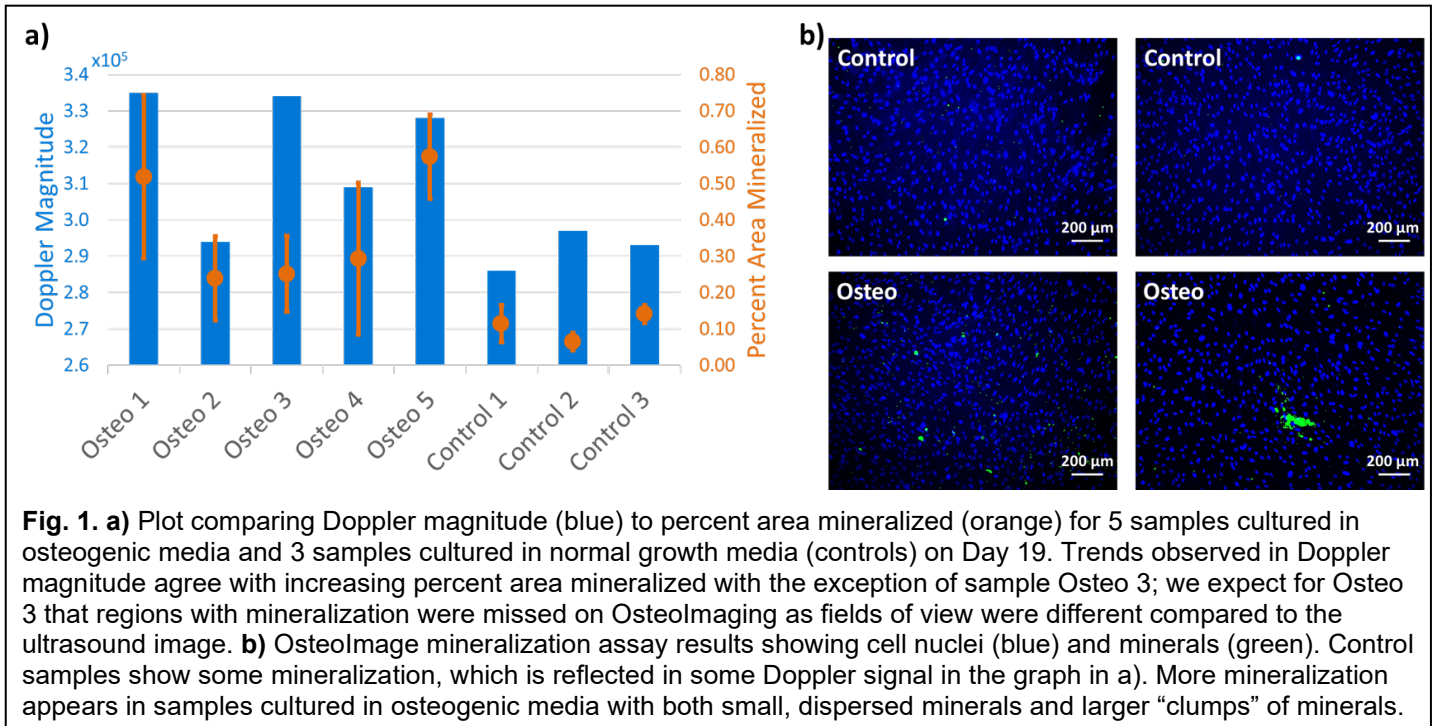
3. ACCOMPLISHMENTS:

o What were the major goals of the project?

1. Evaluate Doppler imaging in ossified and normal human bone marrow-derived stem cells. Target completion: May 31, 2023. Percent completion: 90%
2. Obtain all regulatory approvals for testing ultrasound imaging and therapy in a murine model of heterotopic ossification. Target completion: August 31, 2021. Completion date: November 6, 2020.
3. Evaluate Doppler ultrasound to detect early mineralization in a murine model of heterotopic ossification. Target completion: May 31, 2023. Percent completion: 70%.
4. Evaluate therapeutic ultrasound histotripsy to fractionate minerals and surrounding cells in normal and ossified human bone marrow-derived stem cells. Target completion: June 30, 2023. Percent completion: 75%.
5. Evaluate therapeutic ultrasound histotripsy to fractionate minerals and surrounding cells in a murine model of heterotopic ossification. Target completion: September 30, 2023. Percent completion: 25%.

o What was accomplished under these goals?

1. Evaluate Doppler imaging in ossified and normal human bone marrow-derived stem cells.
 - Lab-grown crystals were used to determine that twinkling was strongest with Doppler ultrasound at 5 MHz, with 12 cycles repeated in 14 ensembles at 3000 Hz and peak acoustic pressures of approximately 2 MPa positive and 1 MPa negative. Of note, these acoustic parameters are the maximum that can be safely achieved with the Verasonics® research ultrasound system and are well within FDA limits of diagnostic ultrasound imaging. These parameters were used to image ossified stem cells every third day from day 10 – day 25. After ultrasound imaging, samples were stained with OsteoImage mineralization assay, and microscopy images captured from three locations across the scaffold for quantification of area mineralized, percent area mineralized, and mean mineralization size. We have completed five rounds of cell imaging. Qualitative results from the OsteoImage mineralization assay shows disperse and “clumped” mineralization in cells cultured in osteogenic media; control samples cultured in growth media show low quantities of disperse mineralization (**Fig. 1b**). Quantitative ultrasound results correlate fairly well with percent area mineralized (**Fig. 1a**); we expect that differing fields of views between OsteoImage and ultrasound imaging explain why the correlation is not perfect in all samples. A



2. Obtain all regulatory approvals for testing ultrasound imaging and therapy in a murine model of heterotopic ossification.
 - IACUC approvals were obtained at the Pennsylvania State University on July 24, 2020. ACURO approval was obtained on November 6, 2020.
3. Evaluate Doppler ultrasound to detect early mineralization in a murine model of heterotopic ossification.

- Heterotopic ossification (HO) was induced in the gastrocnemius muscle of all 15 mice with no adverse events. Mice were imaged with Doppler ultrasound every other day for two weeks, with a subset euthanized after each day of imaging. In some mice we were able to detect HO with Doppler ultrasound as early as day 6, with the signal becoming stronger as HO matured; B-mode images showed morphological changes on day 3 perhaps from the injection protocol (**Fig. 2**). Full quantitative ultrasound processing is ongoing. Samples were processed for histology, but we are still awaiting some chemicals for staining samples. As

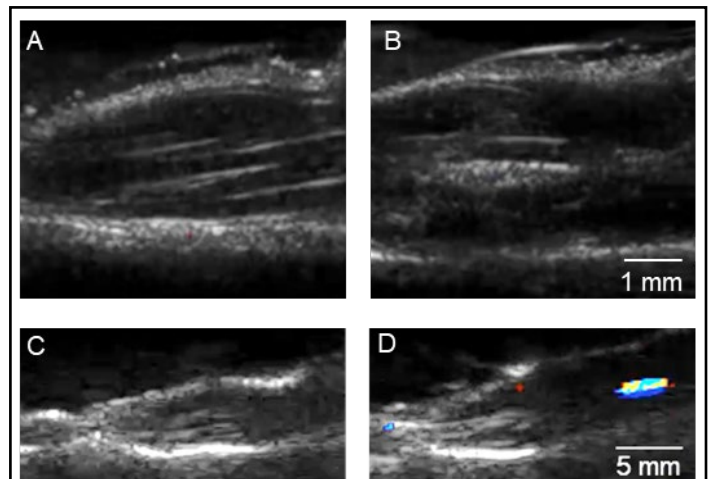


Fig. 2: (A, B) B-mode ultrasound images at 18.5 MHz in the rhBMP4 mouse model. A) Shows the contralateral control leg whereas B) shows the leg injected with rhBMP4 on day 15. A clear compartment of inflammation and HO is visible in the center of the image. This compartment becomes visible 3 days after injection. (C, D) B-mode and Doppler ultrasound images at 5 MHz in the same mouse model on Day 15 showing C) the contralateral control leg and D) twinkling from HO mineralization. This Doppler signal started becoming evident as early as Day 6 in some mouse legs.

soon as chemicals arrive, quantitative ultrasound results will be compared to histological evidence of HO and a manuscript (including data from Task 1) will be prepared for publication.

4. Evaluate therapeutic ultrasound histotripsy to fractionate minerals and surrounding cells in normal and ossified human bone marrow-derived stem cells.
 - In ossified stem cells embedded in a tissue-mimicking phantom, we found treating with 1.07 MHz focused ultrasound with 93 μ s pulses repeated at 20 Hz for four minutes successfully disrupted minerals with minimal damage to the surrounding cells and tissue-mimicking phantom (**Fig. 3**). The corresponding acoustic pressures are 17 MPa peak positive and 6 MPa peak negative, which is similar to the amplitudes found to fragment small, lab-grown crystals. We are currently working on one more round of cells to confirm these results before we will begin preparing an associated manuscript, which may be combined with the results in Task 5.

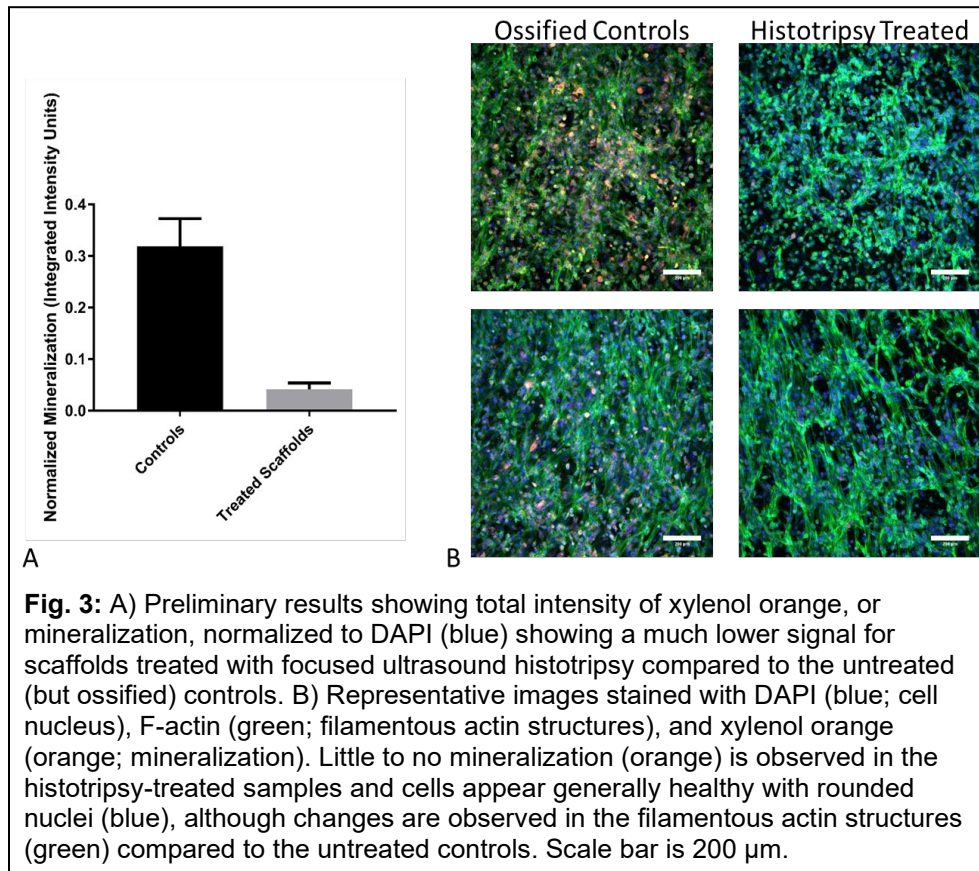


Fig. 3: A) Preliminary results showing total intensity of xylene orange, or mineralization, normalized to DAPI (blue) showing a much lower signal for scaffolds treated with focused ultrasound histotripsy compared to the untreated (but ossified) controls. B) Representative images stained with DAPI (blue; cell nucleus), F-actin (green; filamentous actin structures), and xylene orange (orange; mineralization). Little to no mineralization (orange) is observed in the histotripsy-treated samples and cells appear generally healthy with rounded nuclei (blue), although changes are observed in the filamentous actin structures (green) compared to the untreated controls. Scale bar is 200 μ m.

5. Evaluate therapeutic ultrasound histotripsy to fractionate minerals and surrounding cells in a murine model of heterotopic ossification.
 - We will be inducing HO in 24 mice and evaluating the response to the histotripsy treatment developed and tested in Task 4. We are working to finalize the exact histotripsy parameters for this study with one more round of cell testing. We have all necessary supplies in house and have developed and tested all fixturing necessary to complete the study. Our target completion date is June 2023.
- **What opportunities for training and professional development has the project provided?**
- Fea Morgan-Curtis attended the 182nd Meeting of the Acoustical Society of America in Denver, CO (May 23-27, 2022) and virtually attended the IEEE International Ultrasonics Symposium (October 10-13, 2022) to learn state-of-the field in ultrasound imaging and therapeutics. Fea Morgan-Curtis completed courses in the Graduate Program in

Acoustics at Penn State and received one-on-one mentorship from PI Simon. Lisa Bernsten received one-on-one mentorship from Co-I Hayes.

- **How were the results disseminated to communities of interest?**
 - Imaging results from Tasks 1 and some from Task 3 were presented at the 182nd Meeting of the Acoustical Society of America in Denver, CO (May 23-27, 2022), the Pennsylvania State University College of Engineering Research Symposium (April 6, 2022), and at the IEEE International Ultrasonics Symposium in Venice, Italy (virtual presentation; October 10-13, 2022).
- **What do you plan to do during the next reporting period to accomplish the goals?**
 1. Evaluate Doppler imaging in ossified and normal human bone marrow-derived stem cells.
 - We will finish preparing the manuscript and submit for publication (likely combined with results from Task 3).
 2. Obtain all regulatory approvals for testing ultrasound imaging and therapy in a murine model of heterotopic ossification.
 - Goal is complete.
 3. Evaluate Doppler ultrasound to detect early mineralization in a murine model of heterotopic ossification.
 - We will work to obtain the final chemicals necessary for histological stains Safranin O and Aniline Blue. Once all stains are complete, histological results will be compared to quantitative ultrasound results. Then, a manuscript will be prepared and submitted for publication (likely combined with results from Task 1).
 4. Evaluate therapeutic ultrasound histotripsy to fractionate minerals and surrounding cells in normal and ossified human bone marrow-derived stem cells.
 - We will complete one more round of ossified cells embedded in a tissue-mimicking phantom for confirmation of the results from previous trials. Qualitative metrics from OsteoImage and gross photography will be compared to the ultrasound parameters and a manuscript will be prepared and submitted for publication (likely with data from Task 5).
 5. Evaluate therapeutic ultrasound histotripsy to fractionate minerals and surrounding cells in a murine model of heterotopic ossification.
 - We plan to begin the study in March 2023 with an estimated end date of June 2023 (includes finishing histology staining). Results from this task will be presented at the Acoustical Society of America conference and a manuscript prepared and submitted for publication (likely with data from Task 4).

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
 - We have found that Doppler ultrasound imaging can be used to detect the micro-minerals deposited by cells. This result is novel in that it shows that ultrasound can detect sub-wavelength structures, which opens up a whole new area of untapped potential in diagnostic ultrasound. We expect that microbubbles on the surface of the microminerals allows for detection with Doppler ultrasound. On kidney stones, Doppler ultrasound has been found to detect variations in gas composition and pressure, as these parameters also influence the surface bubbles. This sensitivity of ultrasound to small gas pockets suggests, at some level, that ultrasound can be used to detect changes in metabolism. If proven true, it would allow ultrasound to be leveraged to diagnose a variety of diseases.

- Additionally, the preliminary results from the mouse study suggest that Doppler ultrasound can be used to detect forms of HO earlier than other modalities. Instead of relying on patient-reported symptoms, or ionizing radiation-producing three-phase bone scintigraphy, computed tomography or plain film x-ray imaging, the use of Doppler ultrasound will provide a radiation-free, and thus safer, alternative to diagnose and track HO progression. This advancement in imaging will easily fit within the current standard-of-care for HO, where physicians wait for HO to mature before treatment. With ultrasound, more routine monitoring can be used to track HO maturity and thus enable earlier treatment and reduce sequelae associated with HO.
 - We have found a regime of focused ultrasound histotripsy which can break cell-deposited micro-mineralizations with minimal damage to the cells and the surrounding tissues. We will be testing this regime in a murine HO model to support the *in vitro* results. The results will be especially profound if the use of focused ultrasound histotripsy does not cause recurrence of HO, allowing for immediate treatment of HO, reducing the societal cost of HO, and allowing military service personnel to return to active duty with minimal down time. Even if HO cannot be treated earlier with ultrasound, the benefits of a non-invasive surgery (i.e. lower risk of infection, less tissue trauma, etc.) greatly reduce the societal and military burden of HO.
- **What was the impact on other disciplines?**
 - Our finding that Doppler ultrasound can be used to detect micro-mineralization applies to pathological minerals outside HO including breast microcalcifications, gout, or encrusted catheters/stents. It also may apply to geology for early detection of travertine limestone activity or deposits. Similarly treating HO with focused ultrasound histotripsy may also be adapted for other pathological mineralizations or be used to more effectively destroy mineral build-up on sensitive materials.
 - The possibility that we can extend the results from detecting HO to cellular metabolism would have an even greater impact on a variety of disease. If we can extend the results from detecting ossified stem cells and HO to gases at the cellular level, we could detect earlier forms of a wide variety of diseases. This result has the potential to change the role of ultrasound in medicine.
 - **What was the impact on technology transfer?**
 - Nothing to Report
 - **What was the impact on society beyond science and technology?**
 - Because of the portability and reduced costs of diagnostic and therapeutic ultrasound compared to other imaging and therapeutic modalities, we are reducing societal discrimination and potentially improving the quality of care for those in rural areas. The portability and cost help improve the socioeconomic and location health care discrimination by replacing expensive modalities only offered in large hospitals to cheaper and more portable ultrasonic technologies.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
 - Nothing to Report.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
 - We have been unable to obtain chemicals for safranin O and aniline blue histological stains. We are working with suppliers to obtain the chemicals for these histological stains. If we are unable to obtain them, we will either move forward with reporting results from Task 3 (and perhaps Task 5 depending on the length of the delay) with only the

H&E stain and/or will consult the literature for other alternatives that will allow us to track the progression of HO.

- **Changes that had a significant impact on expenditures**
 - PI Simon's graduate student on the project (Fea Morgan-Curtis) took a medical leave of absence for Fall 2022 semester prompting the need for the no-cost-extension. She is back as of Spring 2023 semester and is excited about finishing the project.
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
 - Nothing to Report.
- **Significant changes in use or care of human subjects**
 - Nothing to Report.
- **Significant changes in use or care of vertebrate animals.**
 - Nothing to Report.
- **Significant changes in use of biohazards and/or select agents**
 - Nothing to Report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**
 - **Journal publications.**
 - Nothing to Report.
 - **Books or other non-periodical, one-time publications.**
 - Nothing to Report.
 - **Other publications, conference papers, and presentations.**
 - Ruge-Jones L, Bernsten L, Morgan-Curtis F*, Hayes D, Simon JC (2022). "Early detection of heterotopic ossification using the color Doppler ultrasound twinkling artifact." IEEE International Ultrasonics Symposium, October 10-13, 2022, Venice, Italy and online. (virtual attendance)
 - Ruge-Jones L, Bernsten L, Morgan-Curtis M*, Hayes D, Simon JC (2022). "Investigating the color Doppler twinkling artifact as an early detector of cellular mineralization." 182nd Meeting of the Acoustical Society of America, Denver, CO USA: May 23 – 27, 2022.
 - Morgan-Curtis FK*, Ruge-Jones L, Berstein L, Hayes D, Simon JC (2022). "Treating calcium phosphate crystals with focused ultrasound." Penn State College of Engineering Research Symposium, University Park, PA, USA: April 6, 2022.
- **Website(s) or other Internet site(s)**
 - Nothing to Report
- **Technologies or techniques**
 - Nothing to Report.
- **Inventions, patent applications, and/or licenses**

- Nothing to Report.
- **Other Products**
 - Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Julianna Simon
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	ORCID ID: 0000-0002-0425-9939
Nearest person month worked:	1
Contribution to Project:	Dr. Simon maintains fiscal and administrative responsibility for the project. As such, she oversees her graduate student, Fea Morgan-Curtis, and meets regularly with her Co-investigator Dr. Daniel Hayes and his graduate student Lisa Bernsten.
Funding Support:	This Award

Name:	Daniel Hayes
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	ORCID ID: 0000-0001-9499-4111
Nearest person month worked:	1
Contribution to Project:	Dr. Hayes supervises his graduate student, Lisa Bernsten, and provide guidance on culturing ossified stem cells. He also participates in project meetings.
Funding Support:	This Award

Name:	Fea Morgan-Curtis
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	4
Contribution to Project:	Ms. Morgan-Curtis helped finish the effort on Doppler imaging of normal and ossified stem cells and imaging of HO-induced mice. She has worked to evaluate histotripsy for treating ossified cells in the tissue-mimicking phantom and is prepared for the murine study evaluating histotripsy treatment of HO in mice. Additionally, she

	presented at three scientific conferences (Acoustical Society of America in Denver, CO, Penn State College of Engineering Research Symposium in State College, PA, and virtually for the IEEE International Ultrasonics Symposium).
Funding Support:	This award with tuition covered by the Graduate School at The Pennsylvania State University.

Name:	Lisa Bernsten
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6
Contribution to Project:	Ms. Bernsten has led the effort in culturing the normal and ossified stem cells for Doppler imaging, including staining and collecting images of the distribution of minerals on the scaffolds for comparison with the ultrasound results. She has also managed the histological samples from the animal studies, including collecting, mounting, and staining.
Funding Support:	This award with tuition covered by the Graduate School at The Pennsylvania State University.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

- **PI Simon:**

- **PROJECTS FUNDED SINCE LAST REPORT**

R01EB023860 NIH NIBIB	Simon	09/2022 – 06/2026	3.0 months
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Focused ultrasound-induced cavitation in elastic, anisotropic tissues: a treatment for tendinopathies

The goal of this project is to develop histotripsy, which is a focused ultrasound modality, to treat injuries to highly collagenous tissues, such as tendon. Aim 1: Assess novel fUS to induce ranges of mechanical fractionation in healthy and tendinopathic *ex vivo* large animal tendons. Aim 2: Integrate PCI and tissue Doppler imaging for quantitative, real-time assessment of fUS therapy. Aim 3: Evaluate fUS to treat chronic tendinopathy in a porcine model.

No number Pennsylvania State University Center for Biodevices oxygen to improve kidney stone detection with ultrasound	Simon	08/2022 – 07/2023 Supplemental	0.12 month
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The goal of this project is to evaluate whether gas content surrounding a kidney stone influences the twinkling artifact. Aim 1: Evaluate whether supplemental oxygen enhances the color Doppler ultrasound twinkling artifact to detect kidney stones. Evaluate the effects of stone and ambient gas compositions on the color Doppler ultrasound twinkling artifact on *ex vivo* human kidney stones. Aim 3: Evaluate whether the color Doppler ultrasound twinkling artifact with supplemental oxygen will allow ultrasound to distinguish Randall's plaques from kidney stones.

- **PROJECTS COMPLETED SINCE LAST REPORT**

R21EB072886 Simon 05/2019 – 10/2022 0.5 month
NIH NIBIB

Histotripsy for collagenous tissues: a novel therapeutic approach to tendon injury
The goal of this project is to demonstrate that histotripsy can emulsify collagenous tissues using tendons as a model. Aim 1: Test novel histotripsy protocols to induce microdamage in collagen gels and ex vivo rat supraspinatus tendons. Aim 2: Evaluate mechanical properties of tendon samples following histotripsy and dry needling and develop a finite element model to evaluate parameters and perform predictive analyses. Aim 3: Determine whether histotripsy enhances collagenous tissue healing in vivo in a pilot survival study of rat tendons.

- **Co-I Hayes:**

- **PROJECTS FUNDED SINCE LAST REPORT**

None to report.

- **PROJECTS COMPLETED SINCE LAST REPORT**

2032582 Hayes 06/2020 – 05/2022 0.09 month
NSF

RAPID: Collaborative Research: One-step express test for presymptomatic detection to prevent COVID-19 spread.

This project aims to develop a rapid, lab-on-chip assay for virus identification.

- **What other organizations were involved as partners?**

- Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

- Nothing to Report

9. APPENDICES:

IEEE International Ultrasonics Symposium abstract (Online participation, October 10-13, 2022)

Early Detection of Heterotopic Ossification Using the Color Doppler Ultrasound Twinkling Artifact

Lucas Ruge-Jones¹, Lisa Berntsen¹, Fea Morgan-Curtis¹, Daniel Hayes¹, Julianna Simon¹

¹Pennsylvania State University, State College, USA

Background, Motivation and Objective

Heterotopic ossification (HO) is the growth of bone in areas of the body where bone does not typically exist. HO can occur after a traumatic injury and causes discomfort and loss of motion which increases the longer HO is left untreated. Three-phase-bone scintigraphy is the most sensitive detector of early HO, but HO foci are not visible until about 3.5 weeks post-trauma, and it exposes patients to ionizing radiation. The Color Doppler ultrasound twinkling artifact is a rapid color shift in the Doppler image that appears on hard, mineral structures in the body, such as kidney stones. It has the potential to be a safe and more sensitive detector of HO. Our objective is to evaluate if the twinkling artifact can detect mineralization in ossified stem cells, an *in vitro* model of HO.

Statement of Contribution/Methods

Human bone marrow stem cells (hBMSCs) were seeded on collagen scaffolds and cultured in osteogenic media (OM) to promote ossification. Five cell samples and three controls (cultured in growth media (GM)) were imaged every third day from day 10 - 25. A Verasonics® Vantage-128 research ultrasound system and three linear transducers (Philips L7-4, L12-5 50mm, and Vermon L22-14v) were used. In phase quadrature (IQ) data and live video were captured for 30 seconds on each cell sample with transducer. Doppler parameters were kept consistent with 14 ensembles with 6-12 cycles repeated at 3000 Hz; Doppler voltages ranged from 25V-

35V. After imaging, the cells were fixed and stained using DAPI (4',6-diamidino-2-phenylindole) and OsteoImage to analyze cell health and mineralization.

Results/Discussion

Twinkling was detected with the L7-4 transducer (5.2 MHz, 12 cycles per ensemble, Doppler voltage of 35V); no twinkling was visible using the other transducers. By day 19, OM samples showed a 0.7 ± 0.2 dB, or approximately 14%, increase in Doppler magnitude compared to the average GM control. Two of the three points that showed substantial increase in the Doppler signal also had mineralization on $0.5 \pm 0.2\%$ of their sampled surface, compared with the average GM value of $0.10 \pm 0.05\%$. This relationship suggests that TA can detect mineralization on the cellular level as early as day 19 and may therefore be a viable detector of HO. [Work supported by DoD CDMRP PR201164].

182nd Meeting of the Acoustical Society of America Abstract (Denver, CO, May 23-27, 2022)

Investigating the color Doppler twinkling artifact as an early detector of cellular mineralization

Lucas Ruge-Jones, Lisa Bernstein, Fea Morgan-Curtis, Daniel Hayes, Julianna C. Simon

Heterotopic ossification (HO) occurs when bone develops in areas where bone does not usually exist, often appearing after blast injuries or other musculoskeletal trauma. Current methods of diagnosis, such as 3-phase bone scintigraphy, x-ray, or CT scans, take several weeks after the trauma before HO is visible. The color Doppler ultrasound twinkling artifact has been shown to detect the presence of kidney stones and other crystals. Twinkling could provide a more sensitive method of HO detection, which would allow for earlier diagnosis and treatment and limit HO severity. In our research, human bone marrow-derived stem cells (HBMSCs) were plated onto collagen scaffolds and cultured in osteogenic media to promote mineralization or growth media (controls). The cells were imaged every 3rd day from day 10 – day 25 using Phillips L7-4, L12-5, and Vermon L22-14v transducers and a Vantage-128 research ultrasound system; I/Q data was bulk saved for quantitative analysis. Samples with mineralization on 0.5% of their surface showed a 17% increase in Doppler magnitude compared to controls, suggesting that the twinkling artifact is a sensitive method for detection of mineralization in vitro. Further studies will investigate whether this method is effective in a murine model. [Work supported by DoD CDMRP PR201164].

Pennsylvania State College of Engineering Research Symposium Abstract (University Park, PA, April 6, 2022)

Treating calcium phosphate crystals with focused ultrasound

Fea K. Morgan-Curtis, Lucas Ruge-Jones, Lisa Bernstein, Daniel Hayes, Julianna C. Simon

Heterotopic ossification (HO) occurs when cells in soft tissue deposit calcium phosphate crystals, or bone, usually due to musculoskeletal trauma. Current treatments of HO include radiation therapy, surgical resection, or shock wave therapy. However, most physicians wait up to 1.5 years after the trauma for HO to mature before starting treatment to reduce the risk of recurrence. Here, we are investigating non-invasive focused ultrasound to fractionate the calcium phosphate crystals and disrupt the surrounding cells as a potential treatment for HO. Calcium phosphate crystals were embedded in collagen or tissue-mimicking polyacrylamide phantoms. Other samples consisted of human bone marrow-derived stem cells (HBMSCs) cultured in osteogenic media on collagen scaffolds. All samples were treated with focused ultrasound at 1.07 MHz, with peak pressures up to $p_+ = 47$ MPa and $p_- = 11$ MPa and pulse lengths up to 10 ms repeated at 200 Hz; total treatment time ranged up to 5 minutes. Results showed that smaller crystals (<1 mm) were successfully fractionated with $p_+ = 12$ MPa and $p_- = 6$ MPa delivered with 18 μ s pulses repeated at 200 Hz for 5 minutes. Experiments with HBMSCs are ongoing. Future studies will test these treatment parameters in mice. [Work supported by DoD CDMRP PR201164].