AWARD NUMBER:	W81XWH-19-1-0168
TITLE:	Quantitative assessment of post-traumatic osteoarthritis by multi-modal optical coherence tomography
PRINCIPAL INVESTIGATOR:	B. Hyle Park
CONTRACTING ORGANIZATION:	The Regents of the University of California Riverside, CA
REPORT DATE:	July 2021
TYPE OF REPORT:	Annual Report
PREPARED FOR:	U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012
DISTRIBUTION STATEMENT:	Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
July 2021	Annual	01Jun2020-31May2021

4. TITLE AND SUBTITLE
Quantitative assessment of post-traumatic osteoarthritis by multi-modal optical coherence tomography

5a. CONTRACT NUMBER
W81XWH-19-1-0168

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Development 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
Evidence from osteoarthritis (OA) studies suggests that there is a narrow time window in the early stages of the disease when cartilage can be functionally restored to reduce further degeneration. Small internal cartilage damages due to traumatic joint injuries are hard to detect with the traditional imaging technologies but pose a significant risk of inducing OA later. Our goal is to develop a non-destructive and label-free combination of optical coherence tomography (OCT) based methods for early detection of PTOA by assessment of mechanical strength, which is dependent upon both GAG and collagen, through our novel method for optical coherence elastography (OCE) based on fringe washout, and of collagen by itself through polarization-sensitive OCT. While our progress has been slowed by the current COVID-19 pandemic, we have demonstrated, for the first time to our knowledge, volumetric assessment of the mechanical properties of cartilage and bone. We anticipate successful completion of our aims as our campus re-opens for research activities. This work will provide the first optical method capable of complete non-destructive assessment of sub-surface cartilage degeneration for PTOA diagnosis.

15. SUBJECT TERMS
post-traumatic osteoarthritis, early detection, cartilage, glycosaminoglycan, collagen, mechanical strength, Young's modulus, optical imaging, optical coherence tomography, optical coherence elastography, polarization-sensitive

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

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1. INTRODUCTION

Evidence from osteoarthritis (OA) studies suggests that there is a narrow time window in the early stages of the disease when cartilage can be functionally restored to reduce further degeneration. These studies collectively demonstrate the importance of early detection of OA to enhance the effectiveness of subsequent therapies. However, current technologies, including arthroscopy, X-ray radiography, and MRI, can detect OA only after significant and irreversible damage to articular cartilage has already occurred. Small internal cartilage damages due to traumatic joint injuries are hard to detect with the traditional imaging technologies but pose a significant risk of inducing OA later. Therefore, it is essential to develop tomographic imaging tools with high resolution that can provide direct assessment of intra-cartilaginous damage in individual patients at the earliest stages of PTOA. We believe that high-resolution assessment of not only the surface, but also the interior portions of cartilage will allow for detection of OA at a much earlier time point, thus providing an opportunity to prevent the progression of or even to allow for repair of cartilage damage and to guide therapies to where they are most effective for existing damage. To take advantage of this therapeutic window, our goal is to develop a non-destructive and label- free combination of optical coherence tomography (OCT) based methods for early detection of PTOA. Cartilage damage at the early stages of OA is known to heterogeneously alter local density/mechanical properties of extracellular matrix (ECM). The mechanical properties of cartilage derive from extracellular matrix components of glycosaminoglycan (GAG) and collagen, with the density and organization of both known to change early in PTOA development. Several investigators have previously established PS-OCT as a method for quantifying localized changes in collagen, but this method is not sensitive to glycosaminoglycan (GAG), which also significantly contributes to the mechanical properties of cartilage. A smaller number of separate studies have investigated the use of optical coherence elastography (OCE) to examine the overall mechanical properties of cartilage, but these techniques cannot provide volumetric quantification in real time. Our novel method for OCE takes advantage of fringe washout, an artifact related to motion during the acquisition time of spectral domain OCT systems, and can be used to rapidly scan volumes of tissue in combination with PS-OCT. Our hypothesis is that robust and sensitive detection of early PTOA can be achieved by utilizing OCE to quantify subsurface damages in GAG loss and its associated cartilage swelling when complemented by quantification of localized changes in collagen content/disorganization with PS-OCT. This project has three aims: 1. optimize fringe washout based OCE, 2. optically quantify GAG in cartilage, and 3. identify optical signatures of cartilage degeneration in early stages of a rat PTOA model. Completion of these aims will provide the first optical method capable of complete non-destructive assessment of sub-surface cartilage degeneration for PTOA diagnosis.

2. KEYWORDS

post-traumatic osteoarthritis, early detection, cartilage, glycosaminoglycan, collagen
mechanical strength, Young's modulus
optical imaging, optical coherence tomography, optical coherence elastography, polarization-sensitive

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1: optimize fringe washout based optical coherence elastography

- Major task 1: relative delay optimization
  - Milestone: autocalibration of optimized PS-OCT/OCT imaging system (month 4)
    - Progress: 100% (previously 100%)
- Major task 2: characterization of sensitivity and resolution
  - Milestone: optimized OCE acquisition parameters based on imaging scenario with expected sensitivity and resolution based on sample size and composition (month 7)
    - Progress: 100% (previously 90%)

Specific Aim 2: optically quantify GAG in cartilage

- Major task 1: bovine cartilage explant experimentation and analysis

- Milestone: calibrated determination of mechanical and biochemical properties of cartilage based on optical imaging (month 10)
  - Progress: 50% (previously 0%)

Specific Aim 3: identify optical signatures of cartilage degeneration in early stages of a rat PTOA model

- Major task 1: early rat OA model experimentation and analysis
  - Milestone: obtain ACURO approval, obtain UCR IACUC approval of protocol amendment (month 5)
    - Progress: 100% (previously 100%)
  - Milestone: identification of early PTOA based on optical assessment of changes in cartilage (month 18)
    - Progress: 50% (previously 10%)

What was accomplished under these goals?

Specific Aim 1: Hardware modification – PS-OCT

Our imaging system previously used a galvanometer-based scanning system with two single-axis scanning galvanometer mirrors to steer the OCT beam laterally over a sample of interest. We suspect fraying of a wire between one of the drivers and its corresponding galvo led to the galvos becoming unable to hold static position with an uncontrolled vibration along the fast-scanning direction. Due to the age of the unit, we were unable to repair the galvo system, and so were forced to replace the scanners with a single micro-electro-mechanical system (MEMS) based mirror (Mirrorcle) capable of 2-dimensional steering. The new scanner allowed for simplification of the sample arm setup, and this revised setup was used to acquire all PS-OCT data in this report.

Specific Aim 1: System development – OCE

Our method for OCE involves comparison of depth profiles acquired sequentially with and without ultrasound perturbation of the sample. Depth profiles acquired during ultrasound perturbation will have fringe washout, an OCT imaging artifact in which movement of the sample results in decreased signal-to-noise ratio. As the magnitude of localized vibration is dependent upon the mechanical stiffness of the sample, localized measures of the mechanical properties of the sample can be quantified through simple calculation of the amount of fringe washout. OCE data can then be obtained by acquiring depth profiles such that the even- and odd-numbered depth profiles are recorded with and without, respectively, ultrasound and then calculated simply by subtracting the SNR of nearby pairs of depth profiles.

Over the last year, we have been able to refine this basic methodology and remove several imaging artifacts that we observed. In the last progress report, we included OCE data rapidly obtained over a volume of rat knee cartilage. However, upon more careful analysis, we realized that the depth profiles obtained during what we assumed were not affected by ultrasound did in fact suffer from some fringe washout. Further analysis revealed that we were applying too great an intensity of ultrasound perturbation, and that there was residual vibration occurring that resulted in this artifact (example shown in Figure 1). We then did a careful analysis to determine the maximum ultrasound duration and power for different sample types in order to ensure that this artifact is eliminated. In short, this involves acquisition of an image with no ultrasound perturbation whatsoever, such that a t-test over a selected region in the image can be done to compare image characteristics to a composite image formed from the depth profiles assumed to have no ultrasound perturbation in our OCE acquisition mode. A range of delays from 0 to 100 μs were tested for a range of ultrasound durations from 25 to 100 μs in alternating depth profiles were tested for agar tissue phantoms, bovine explant samples, and cartilage on the femur and tibial sides of rat knees were tested. Parameters were selected to identify the ultrasound parameters that yielded a $p > 0.05$ between a composite image formed of depth profiles assumed to have no ultrasound and an image acquired with no ultrasound that also still yielded the greatest magnitude of fringe washout in the ultrasound focal volume. This has resulted in OCE measurements that better correlate with mechanical strength.

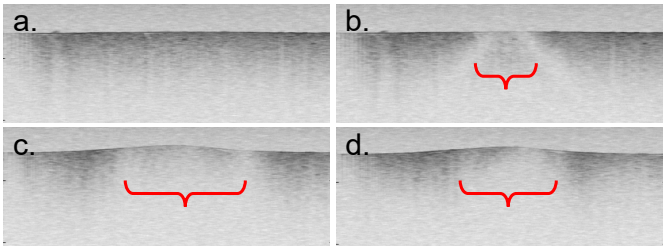

Figure 1: Decomposed images of even depth profiles acquired without ultrasound perturbation (a and c) and of odd depth profiles acquired with ultrasound perturbation (b and d) for ultrasound durations of 50 μs (a and b) and 100 μs (c and d) with a line exposure time of 100 μs. Regions with visible fringe washout are highlighted with the red brackets, and shows an example where residual ultrasound effects can still be detected in depth profiles meant to have no fringe washout.

Specific Aim 2: bovine cartilage explants

Cylindrical cartilage samples having a diameter of 6 mm and a thickness of approximately 2 mm were excised from a bovine femur (Sierra for Medical Science) at the femoral grove using a biopsy punch (Miltex). Cartilage samples were subjected to either 1 mg/mL collagenase type 1 (Worthington) solution for 2, 4, 8, and 24 hours to acquire samples with different degrees of collagen degradation or 2 unit/mL chondroitinase ABC (C-ABC, Sigma) solution for 0.5, 1, 2, and 4 hours to acquire samples with different degrees of GAG degradation. The collagenase type 1 or C-ABC treated samples were kept in PBS with 0.05% of sodium azide and stored at 4 °C until further analysis. Biochemical analysis will be performed after completion of imaging.

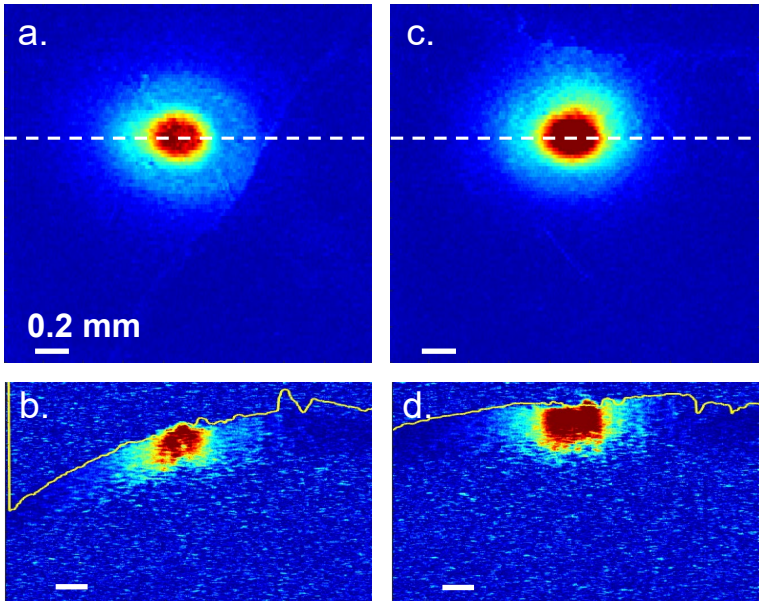

Figure 2: representative OCE data acquired from control (left panels) and C-ABC treated (right panels) bovine explant samples. The two upper images (a and c) are en face reconstructions of the imaged region based on the average of the OCE signal within the first XXX pixels from the tissue surface. The lower two images (b and d) show cross-sectional OCE images from the location indicated by the dotted lines on a jet color scale, where blue indicates less fringe washout and red indicates greater fringe washout.

Specific Aim 2: OCE imaging of samples

The cartilage samples were scanned under the OCE sample arm probe with a scanning line rate of 5 kHz. The camera exposure time was set to be 100 μs during each acquisition of A-line. The lateral imaging range was governed by a XY-galvanometer in the OCT probe. A volumetric scan that covers a square region of 2.4 mm by 2.4 mm on the cartilage surface was performed on each sample. Each volume of data consists of 100 frames of 1024-line cross-sectional images with depth information of 2 mm.

During the scanning, the ultrasound transducer was synchronized with OCT acquisition, and triggered by the line trigger signal sent at every other A-line acquisition (ultrasound pulse repetition frequency: 2.5 kHz). Each pulse of the ultrasound signal was modulated by sinusoidal waves at the transducers resonance frequency of 6.8 Hz with peak-to-peak voltage of 300 mV. The signal was then amplified by an amplifier (Gain 55 dB) before sending to drive the ultrasound transducer. We controlled the ultrasound perturbation power by adjusting the duration of each ultrasound pulse depending on the sample stiffness. The duration of each ultrasound pulse was set to 50 μs on the bovine cartilage samples

Figure 2 shows representative OCE data acquired from control (left panels) and C-ABC treated (right panels) bovine explant samples. The two upper images (a and c) are en face reconstructions of the imaged region based on the average of the OCE signal within the first XXX pixels from the tissue surface. The lower two images (b and d) show cross-sectional OCE images from the location indicated by the dotted lines on a jet color scale, where blue indicates less fringe washout and red indicates greater fringe washout. The lateral area of the ultrasound focus is evident in the en face images. The difference in OCE magnitude is quite clear; both the area and intensity in the cross-sectional OCE images is smaller and weaker in the control sample (9.231±0.871 dB) compared to the sample treated with C-ABC (11.407±0.715 dB). This is consistent with expectations, as GAG degradation should lead to decreased mechanical strength, which in turn leads to greater fringe washout.

Specific Aim 3: Induction of PTOA

Impact loadings were applied to the femorotibial joint of rats after institutional approval (UCR IACUC #20190022). 12-week old rats were anesthetized and placed under a custom-designed drop tower. The right knee was bent at approximately 45 degrees and placed in a position where the cartilages on medial, lateral condyles and the patella above the femoral grove was directly underneath the drop mass. Different weights of drop mass were used to apply 0.2, 0.5, and 1 J of impact energy to the knee based on the calibration curve (Figure). A total of six rats (2 rats/condition) were used in this experiment and contralateral knees were used as controls.

The rats were sacrificed at 2-week post-impact. The samples (femurs and tibias) were harvested and fixed in formalin for 48 hours. The formalin was then replaced with PBS with 0.05% of sodium azide and the samples were stored at 4 °C until further analysis.

Specific Aim 3: OCE imaging of rat cartilage

These samples were imaged with a similar procedure to that described above for the bovine explant samples, with the exception that an ultrasound pulse duration of 100 μs was used.

Figure 3 shows representative OCE data acquired from control (left column) and injured (right column) femurs. In the 650 nm range, cartilage has greater backscatter than bone, and so the upper (solid yellow) and lower (dotted yellow) boundaries of the cartilage can be identified using intensity thresholding of filtered cross-sectional images. En face reconstructions based on the average OCE signal within the cartilage are shown in the bottom row (c and f; jet color scale). Quantification of the magnitude of the fringe washout within the ultrasound focus for the control and injured samples was 9.414±1.227 and 7.858±1.115 dB, respectively, which is consistent with expectation for damaged cartilage to have a higher Young's modulus and thus show less fringe washout.

Specific Aim 3: PS-OCT imaging of rat cartilage

Acquisition of volumetric PS-OCT data has been completed for the 6 preliminary rats prepared as described above. All samples were imaged with a depth scan line rate of 2 kHz and a line exposure time of 0.2215 ms. The scanning parameters used for femurs utilized 100 cross-sectional images spanning 4.5 mm, with each image composed of 1024 depth scans over 4.5 mm. Due to the larger physical size of the end of the tibia, scanning parameters were expanded to 6.75 mm in each direction.

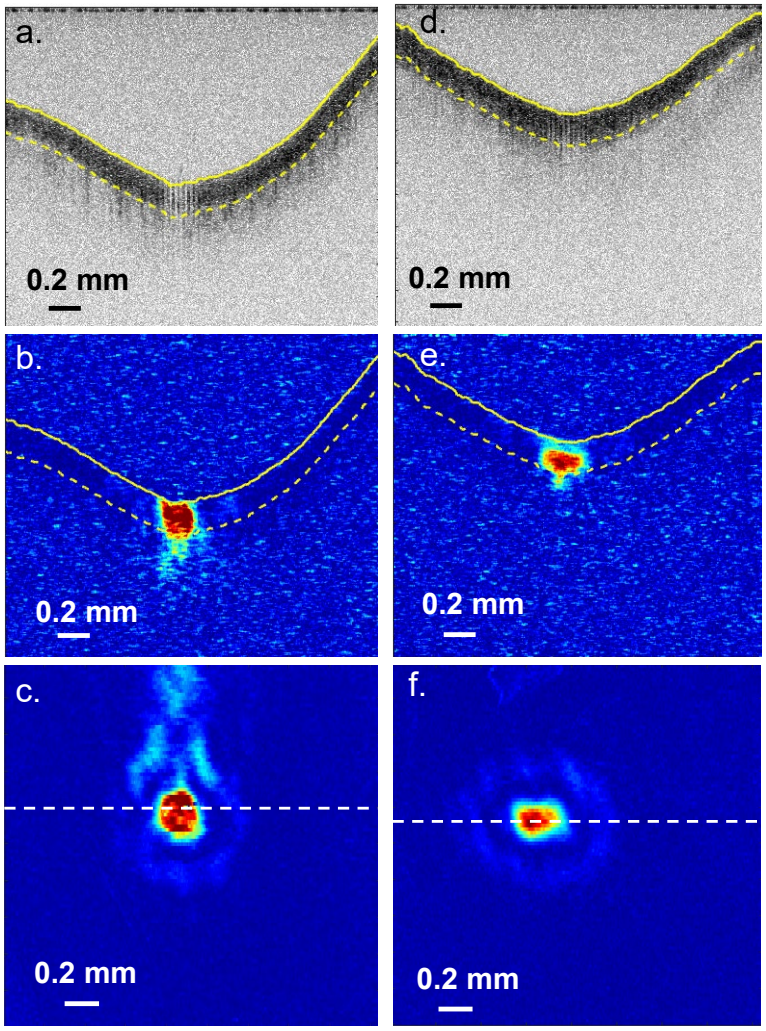

Figure 3: representative OCE data acquired from control (left column) and injured (right column) femurs. Upper (solid yellow) and lower (dotted yellow) boundaries of the cartilage are overlaid on cross-sectional images (a and d; logarithmic gray-scale). Corresponding OCE images are shown in the middle row (b and e; jet color scale). En face reconstructions based on the average OCE signal within the cartilage are shown in the bottom row (c and f; jet color scale).

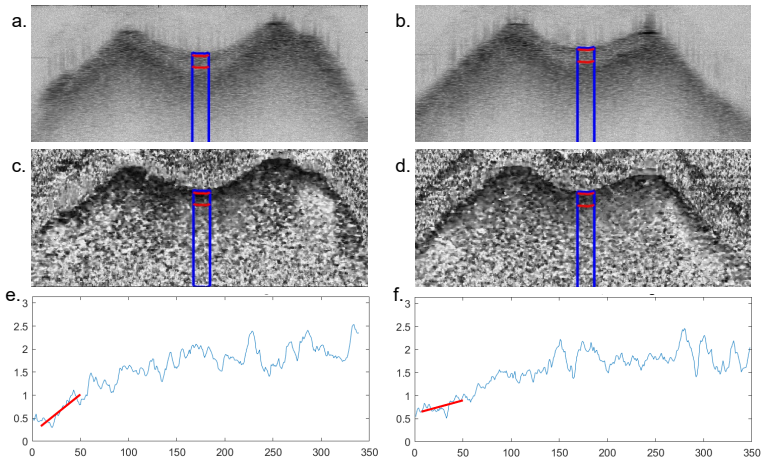

Figure 4: PS-OCT data from the femoral cartilage of a control (left) and an injured (right) rat knee. Cross-sectional OCT intensity images (1024 depth profiles spanning 4.5 mm) are shown in the top row; double-pass phase retardation images are shown in the middle; plots of the average DPPR versus the depth below the cartilage surface are shown at the bottom.

Figure 3 shows representative results of PS-OCT data acquired from the cartilage at the end of control and injured femurs. In the 1310 nm range, cartilage has less backscatter than bone, and so the upper and lower boundaries of the cartilage can be identified through intensity thresholding of filtered cross-sectional images (horizontal red bands). We anticipate cartilage damage to be localized to small regions in these samples, and so the average birefringence, calculated as the fitted slope of the double-pass phase retardation (DPPR) per unit depth within the cartilage (red fit line), is determined for a moving window (vertical blue bars). Preliminary

analysis of the DPPR over the lateral condyte, in the patella groove, and over the medial condyte from the 6 rats yields 2.38, 3.25, and 2.80 degrees/pixel. A significant decrease in cartilage birefringence in the injured sample can be quantified through the fitted slope of the DPPR within the cartilage in the patella groove for the control and injured samples are 4.298 and 1.460 degrees/pixel, respectively. A comparison of the control and injured femur samples shown in Figure 3 demonstrates to detect a significant decrease in the birefringence in the patella groove of the injured sample on the right column, which we believe is due to PTOA-related disorganization of the collagen network in the cartilage.

What opportunities for training and professional development has the project provided?
Nothing to report.

How were the results disseminated to communities of interest?
Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
COVID-19 led to an unexpected and lengthy delay on this overall project. While limited and restricted access to our laboratories was permitted, we encountered massive delays over the past year. Most of these restrictions have been eased, particularly over the month or so, and based on our current level of access to our laboratories and other resources, we are planning to request an extension of the project period and expect to be able to complete the goals of the project within the next reporting period. In particular, we plan to complete the following:

- Bovine explant experiments: Preliminary optical scanning of the samples prepared so far has been completed, but a significant amount of data processing is still pending. Upon completion of this analysis, the samples will be subjected to biochemical analysis. It was noted that the samples prepared so far displayed visible changes in mechanical strength (i.e., a loss of structural integrity was evident upon visual inspection). We therefore anticipate the need to develop a more graded loss of mechanical strength in additional bovine explant samples in order to extract a statistically significant correlation between optical-derived measures and biochemical analysis of collagen and GAG content.
- PTOA induction and analysis: The relaxation of COVID-related restrictions recently has allowed us to complete a preliminary test run of the 6 animals described in this report. Upon completion of data analysis of the acquired optical imaging data, the sample will be subjected to histopathological analysis. Analysis of the combined data will inform guide preparation of upcoming experiments (e.g., impact loading, better aiming of the drop tower onto the knee, development time for PTOA) for completion of the proposed work.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
We anticipate that, upon completion of analysis of these results, we will be able to provide the first demonstration, to the best of our knowledge, of the ability to non-destructively assess loss of collagen structure and of GAG structure in cartilage in PTOA. We expect to achieve this based on our preliminary results that at this point confirm that OCE can detect changes in mechanical strength due to a loss of both collagen and GAG structure, and that PS-OCT largely quantifies loss of collagen. The impact of our current progress opens the possibility of non-destructive assessment of volumetric mechanical properties of cartilage as a method for early detection of PTOA onset and progression.

What was the impact on other disciplines?
Nothing to report.

What was the impact of technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

5. CHANGES / PROBLEMS

Changes in approach and reasons for change
No changes to report.

Actual or anticipated problems or delays and actions or plans to resolve them
We were overly optimistic in last year's progress report regarding resumption of research activities, as COVID-related restrictions and delays over the past year proved to be far more significant that could have been predicted. While it has taken quite a bit longer than originally anticipated, regulations have been relaxed on campus, especially over the last month or so, such that research activities can finally resume as planned. We anticipate requesting an extension of the project period to complete the proposed work.

Changes that had a significant impact on expenditures
No changes to report.

Significant changes in use or care of human subjects
N/A.

Significant changes in use or care of vertebrate animals
No changes to report.

Significant changes in use or biohazards and/or select agents
N/A.

6. PRODUCTS

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	B. Hyle Park
Project Role:	PI
Research Identifier:	https://orcid.org/0000-0002-0282-7162
Nearest person month worked:	3
Contribution to project:	Supervision of overall project progress through weekly meetings

Name:	Jin Nam
Project Role:	PI
Research Identifier:	https://orcid.org/0000-0001-5117-8958
Nearest person month worked:	3
Contribution to project:	Supervision of overall project progress through weekly meetings

Name:	Junze Liu
Project Role:	Technician / Graduate student
Research Identifier:	https://orcid.org/0000-0001-7946-4833
Nearest person month worked:	12
Contribution to project:	Design and construction of OCE system; optimization of processing method to visualize tissue mechanical properties

Name:	Youyi Tai
Project Role:	Graduate student
Research Identifier:	https://orcid.org/0000-0002-2530-4225
Nearest person month worked:	12
Contribution to project:	Preparation of bovine explant samples, perform drop tower injuries, preparation of animal models

Name:	Thamidul Islam
Project Role:	Graduate student
Research Identifier:	https://orcid.org/0000-0002-8430-1104
Nearest person month worked:	6
Contribution to project:	Polarization-sensitive OCT data acquisition and analysis

Name:	Luyang Yu
Project Role:	Graduate student
Research Identifier:	
Nearest person month worked:	3
Contribution to project:	OCE data acquisition and analysis

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

There have been no changes to the active other support for the PD/PI(s).

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

N/A.

9. APPENDICES

N/A.