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TITLE: **Development of 3D printed Ophthalmic Tissue for Surgical Training**

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CONTRACTING ORGANIZATION: The Geneva Foundation

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14. ABSTRACT

The main focus of this effort is to develop a platform of 3D printed tissues with intrinsic motion tracking for application in ophthalmic surgical training programs utilizing three state-of-the-art construction methods: electrospinning, 3D bioprinting and BioLP laser induced cell and particle transfer. The proposed simulation training system would combine the strengths of both mechanical and virtual models: a mechanical tissue with a three-dimensional nano- and micro-structure built to the specific known parameters of human tissues with embedded sensors to track tissue manipulation and localized stress and strain during procedures.

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1. INTRODUCTION:

The Accreditation Council for Graduate Medical Education now recommends surgical skills development resources such as wet labs or simulators as a critical benchmarking and basic skills acquisition tool for surgical trainees. Wet lab training scenarios include animal courses (Triservice Ocular Trauma Course), wet lab skills training such as suturing pig eyes, and suturing tissue with similar mechanics, such as a pig foot. Computer virtual simulators such as the Eyesi provide excellent procedural training but lack proper tactile sensation needed for microsurgery and are cost prohibitive. Mechanical training systems such as the Phak-i Surgical Practice Eye and Kitaro Eye allow for affordable practice of cataract removal but the plastic and rubber eyes lack the proper mechanical properties to provide trauma surgical practice and lose the procedure assessment capabilities of virtual systems. Currently, there is no simulation resource, virtual, mechanical, or live, that provides standardized ideal tissue mechanical characteristics, measurable and reproducible trainee tasks, and formative feedback to assess trainee progression in ophthalmologic wound repair. We propose to develop a platform of 3D printed tissues with intrinsic motion tracking for application in ophthalmic surgical training programs utilizing three state-of-the-art construction methods: electrospinning, 3D bioprinting and BioLP laser induced cell and particle transfer. The proposed simulation training system would combine the strengths of both mechanical and virtual models: a mechanical tissue with a three dimensional nano- and micro-structure built to the specific known parameters of human tissues with embedded sensors to track tissue manipulation and localized stress and strain during procedures.

2. KEYWORDS:

Surgical Simulation, Bioprinting, Sensor Array, Electrospinning, 3D Printing, Additive Manufacturing, Medical Education, Motion Tracking

3. ACCOMPLISHMENTS:

The PI is reminded that the contract organization is required to obtain prior written approval from the USAMRAA Contract s Officer whenever there are significant changes in the project or its direction.

- What were the major goals and objectives of the project?
- What was accomplished under these goals?
- What opportunities for training and professional development did the project provide?
- How were the results disseminated to communities of interest?
- What do you plan to do during the next reporting period to accomplish the goals and objectives?

What were the major goals of the project?

Below are listed the major goals of the project as stated in the approved SOW. The specific aims are listed below in outline form. The progress of each aim and sub-aim is identified as the percentage of completion of each task number in the adjacent table. The target work times, and completion are shown for each task of the project as gray blocks in each corresponding quarter. The no cost extensions are indicated with an * (first extension) and a # (second extension). There have not been significant changes in approach or methods from the agency approved application or plan.

Specific Aim 1: Successfully utilize 3D bioprinting technologies to create a critical component of a cost-effective and realistic simulated tissue corneal and scleral wound repair simulator system.

1.1. 3D placement of electrospun collagen lamella.
  1.1.1. Assemble electrospun apparatus with 3D positioning
  1.1.2. Electrospin Collagen fibers of nano- and microscale size
  1.1.3. Electrospin individual fibers into lamellae with or without nanopositioner orientation and determine Young's Modulus
  1.1.4. Electrospin collagen fibrils with Adept robot 3D positioning to form ophthalmic constructs and determine Young's Modulus

1.2. Direct- write 3D bioprinting of Gel MA and crosslinking compounds.
  1.2.1. Acquire and commission 3D bioprinter
  1.2.2. Demonstrate 3D deposition of hydrogels onto electrospun collagen
  1.2.3. Demonstrate crosslinking of electrospun collagen and 3D bioprinted hydrogels and determine Young's Modulus

1.3. 3D printing of living cells

1.3.1. Deposit living cells with BioLP or 3D BioLP/LIFT bioprinter onto culture dish
1.3.2. Deposit living cells into Gel MA gel matrix
1.3.3. Deposit living cells into 3D formed ophthalmic constructs

1.4. Implementation of ophthalmic construct fabrication on a spherical surface
1.4.1. Design 2 rotational degrees of freedom supporting sphere
1.4.2. Fabricate rotating support sphere and integrate with 3D printer, BioLP and electrospray
1.4.3. Demonstrate deposition of ophthalmic constructs on spherical surface

Specific Aim 2: Successfully design, fabricate and 3D print microscale tracking units to provide a surgical motion and intrinsic tissue response to manipulation recording component as an integral part of the surgical simulation system.

2.1. Design and fabrication of wireless microchips for tracking
2.1.1. Design, assemble and evaluate FPGA based circuits for wireless nodal communication
2.1.2. Transfer FPGA design to ASIC microchips with bonded LEDs and photodiodes
2.1.3. Evaluate microchip array read out and modify design as needed
2.1.4. Fabricate custom microchips with incorporated optoelectronics using MOSIS institute
2.1.5. CDRL preparation for Task 2.1

2.2 Precision 3D placement of microscale tracking units using BioLP based method
2.2.1 Deposition of 20, 40 and 100 micron microspheres and microchips into gel structures
2.2.2 Quantification of depth of penetration into gel structures and accuracy of placement
2.2.3 Quantification of deposition into collagen/gel ophthalmic constructs
2.2.4 Fabrication of BioLP printer head at Walter Reed

2.3 Development of wireless microchip tracking system
2.3.1 Design control unit for wireless readout of positional data from nodal sensor array
2.3.2 Fabricate FPGA based control unit and with software for readout and position tracking
2.3.3 Readout data from microchip array imbedded in gel matrix to user interface
2.3.4 CDRL preparation for Task 2.3

2.4 Development of an optically based microsphere tracking system
2.4.3 Design and acquisition of camera based optical particle tracking system and software
2.4.4 Demonstration of particle location and tracking on dry surface
2.4.5 Demonstrate particle location and tracking in gel matrix
2.4.6 CDRL preparation for Task 2.4

Specific Aim 3: Successful integration of 3D bioprinted scleral and corneal tissue with intrinsic tissue motion tracking to a pressurized surgical training system used to standardize GME surgical training modules.

3.1 Development of the tracking system and surgical interface
3.1.1 Procurement and software integration of Pulhemus surgical instrument tracking system
3.1.2 Fabrication of tissue holder with integrated tracking electronics
3.1.3 Completion of software for surgeon user interface and functional testing
3.1.4 Fabrication and assembly of multiple copies of the tissue holder
3.1.5 CDRL preparation for Task 3.1

3.2 Surgical evaluation and collection of data for standardized nomogram.
3.2.1 Assembly of completed device with synthetic tissue and tracking system
3.2.2 GME trainee testing on three wound scenarios at Tri Service Ocular Trauma Course
3.2.3 Assessment of GME performance using modified OSATS scoring criteria with live action and video recordings
3.2.4 Ophthalmology Program Director survey of system utility in training

3.3 Delivery and revisions of CDRLs A001-A009 for all Tasks

Task Number		FY 1				FY 2				FY 3				FY4				FY5			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1			
		Task 1: Simulated tissue for corneal and scleral wound repair simulator																			
1.1.1	100%																				
1.1.2	100%																				
1.1.3	100%																				
1.1.4	100%																				
1.2.1	100%																				
1.2.2	100%																				
1.2.3	100%																				
1.3.1	100%																				
1.3.2	100%																				
1.3.3	100%																				
		Task 2: Design, fabricate and print tracking units																			
2.1.1	100%																				
2.1.2	100%																				
2.1.3	100%																				
2.1.4	100%													*	#	#	#				
2.1.5	90%														#						
2.2.1	100%																				
2.2.2	100%																				
2.2.3	100%																				
2.2.4	100%																				
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2.3.4	90%																				
2.4.1	100%																				
2.4.2	100%																				
2.4.3	100%																				
2.4.4	100%																				

Task Number		FY 1				FY 2				FY 3				FY4				FY5			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1			
		Task 3: Integration of 3D bioprinted tissue with motion tracking units																			
3.1.1	100%																				
3.1.2	100%																				
3.1.3	90%												*								
3.1.4	90%													*	#	#	#	#			
3.1.5	90%												*								
3.2.1	80%													*	#	#	#	#			
3.2.2	50%													#	#	#	#	#			
3.2.3	50%													#	#	#	#	#			
3.2.4	50%											#	#	#	#	#	#	#			
3.3	90%													*	#	#					

What was accomplished under these goals?
For this reporting period (Year 4) describe major accomplishments:

The summary of major accomplishments for each Specific Aim is as follows:

- Aim 1: We refined our spinning technique for longer duration 12 hour production prints and worked on electrospinning onto wet gel surfaces to allow for a single printer gel and spinning process. We developed our multimodal 3D printer to combine gel and electrospin printing from .STL CAD (computer aided design) files standard to the industry.
- Aim 2: We developed 3D particle tracking functions with MATLAB and performed extensive work to demonstrate wireless communication using the mm scale chips. We demonstrated integration with FPGA mini-computer based chip tracking system. Our microchip array was able to detect real-time insertion and removal of chips via data collecting in MATLAB. We prepared data and methods descriptions for CDRL submission and revision.
- Aim 3: We continued to refine tissue fabrication to improve surgical "feel" based on surgeon feedback from our 2019 ocular trauma course study. We prepared for the 2021 ocular trauma course after the cancelation of the 2020 course. We integrated instrument tracking data from the Pulhemus system into MATLAB for display.

Specific Aim 1
1) Major Activities (Accomplishments)

We refined our spinning technique for longer duration 12 hour production prints and worked on electrospinning onto wet gel surfaces to allow for a single printer gel and spinning process. We developed our multimodal 3D printer to combine gel and electrospin printing from .STL CAD (computer aided design) files standard to the industry.

2) Specific Objectives

All the tasks under Specific Aim 1 have been completed in past years and summarized in those annual reports. In this fourth year our objectives related to improving existing methods developed under Specific Aim 1. In particular, we needed to improve the tissue constructs to make them more durable under needle insertion and knot tying. In the past we have been limited in the number of layers of fibers we could produce because we had to move the tissue construct between the electrospinning printer and the gel printer. We put significant effort into making the system more functional to create a multimodal printer that can vary the fiber placement within the construct.

- Complete robot based printer with combined gel and electrospinning.
- Create software to take standard CAD files of the tissue construct and create printer control code.
- Explore options for electrospinning onto damp or wet gel layers to reduce hours of drying time.

3) Significant Results from Year 4

Robot based printing of gel and electrospun fibers

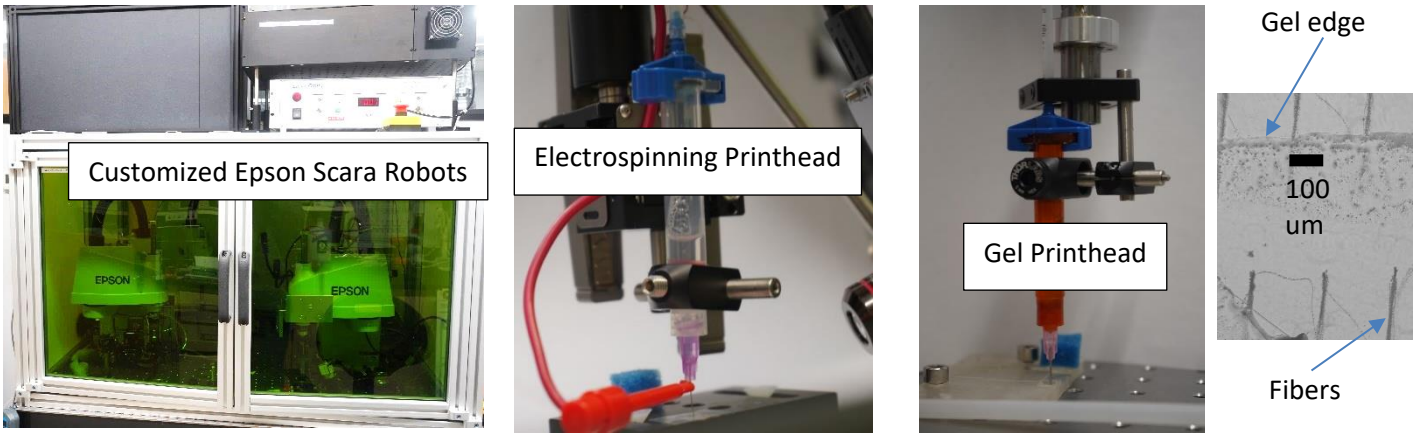


Figure 1. Combined printer.

We have successfully combined both an electrospinning printhead and a gel printhead in one printer. Figure 1 shows the Epson Scara printers used and the two printheads. Both printheads use syringes with blunt tipped needles. The robot arms move the respective printheads over the stage for printing and back to their base cleaning stations. The electrospinning printer includes a high voltage clip and 2 piezo crystal stacks. The piezo crystal stacks allow for oscillatory motion over 1.5mm in x and y. This allows for rapid printing of hundreds of fiber lines per second. We had to develop several automatic calibration methods to align both printheads prior to each run. We also developed realtime imaging to collect data to determine the accuracy of the prints. Figure 2 shows one test run of increasing electrospinning print oscillation speed.

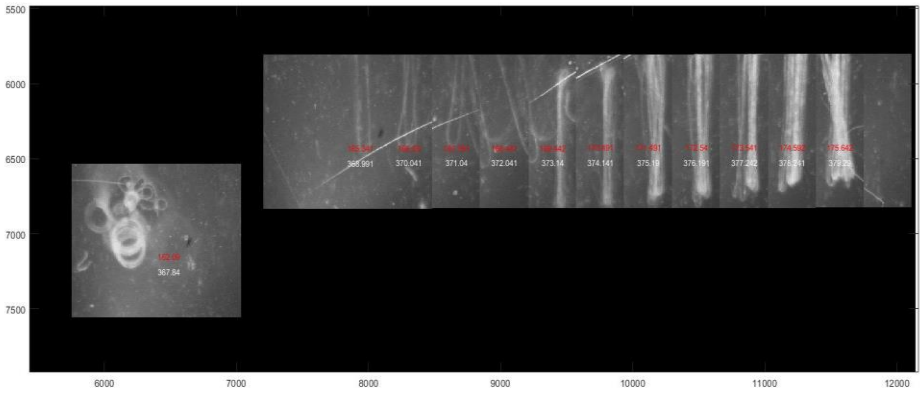


Figure 2. Print test run. The electrospinning printhead was evaluated at different speeds increasing right to left. The leftmost square shows the waiting area where the print head moves to wait until proper fiber formation occurs then it automatically moves to print according to the coded instructions.

Converting CAD .stl files into 3D printer gcode

Once we developed the ability to print both gel and fiber accurately we realized we needed a new method of converting .stl CAD files of the corneas into gcode to control the printer. The gcode is a list of position and gel (or plastic) extrusion instructions that all 3D printers use. The generic name for the software to convert a 3D CAD file to gcode is a slicer. The slicer slices the solid CAD file into layers of preset thickness and determines the path the print tip will follow. The path usually includes and outline for the layer and the infill. For our gel printer could use a standard approach for slicing. However the use of two printheads of different print line thickness required a different approach. We develop a slicer program called WaveSlicer to allow for different layer thicknesses for gel and fiber prints. In addition the electrospun fibers must be laid down hundreds at a time with only 1.5mm maximum length. Figure 3 shows the .stl file and the resulting gel and fiber paths.

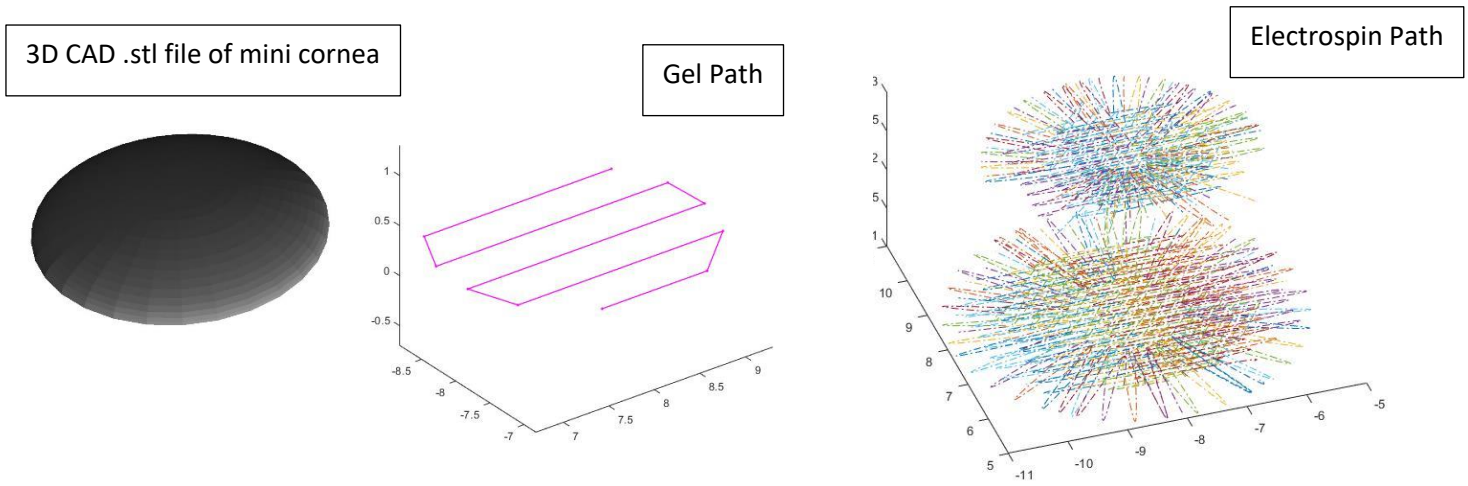


Figure 3. The original CAD file of a sample 3mm "mini" cornea. The gel path assumes 500 micron wide gel extrusions. The electrospin path assumes ~ 1 micron fibers placed with rapid oscillations perpendicular to the path. In addition, the fiber layers are above and below the gel layer.

Wet electrospinning methods search

Problem: Collagen fibers direct-write electrospun directly onto freshly bioprinted and UV-crosslinked gelma layers either partially or completely dissolve when they reach the gelma surface and completely dissolve when additional gelma layers are printed on top of the fibers.

Solution: Evaluate how to incorporate wet spinning buffer (taken from wet spinning technique described here) into electrospun cornea as a collagen fiber stabilizer.

Plan A

-Mist/spray wet spinning buffer solution directly onto freshly bioprinted and UV-crosslinked gelma prior to direct-write electrospinning of collagen

-Print a container that holds a reservoir of wet spinning buffer in contact with printed gelma layer

Plan B

-Mix wet spinning buffer into GelMa solution from advanced biomatrix and see if gelma gels normally

-Wet spinning buffer (coagulation buffer) is made of:

10% PEG (10g in 100mL);

4.14 mg/ml monobasic sodium phosphate→ 414 mg;

12.1 mg/ml dibasic sodium phosphate→ 1,210 mg;

6.86 mg/ml TES→ 686 mg;

7.89 mg/ml Sodium chloride → 789 mg;

Figure 4. Fiber mats spun using wet buffer.

Specific Aim 2

1) Major Activities (Accomplishments)

We developed 3D particle tracking functions with MATLAB and performed extensive work to demonstrate wireless communication using the mm scale chips. We demonstrated integration with FPGA mini-computer based chip tracking system. Our microchip array was able to detect real-time insertion and removal of chips via data collecting in MATLAB. We prepared data and methods descriptions for CDRL submission and revision.

2) Specific Objectives

Our specific objectives are found in the subtasks listed below. All tasks under Specific Aim 2 were completed in past project years and included in those reports except for Tasks 2.1.4 and 2.1.5. These tasks have been completed in quarter 16 as seen in the SOW Table above.

2.1.4 Fabricate custom microchips with incorporated optoelectronics using MOSIS institute

- We fabricate the chips in year 3 but needed to test and package the devices for evaluation.
- We needed to use the FPGA minicomputer to connect to an array of chips and bring the network data into a standard PC to demonstrate observation of movement in the network

2.1.5 CDRL preparation for Task 2.1

- We needed to submit and revise the CDRLs and associated data and methods descriptions.

3) Significant results from project year 4 focusing on Tasks 2.1.4 and 2.1.5.

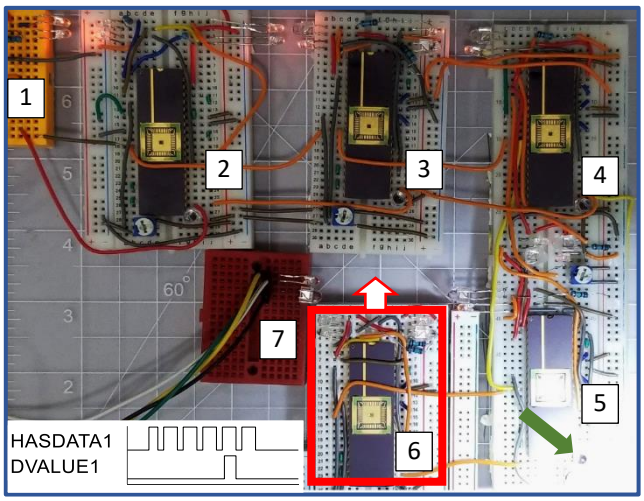
Tracking real time motion with array of optically communicating microchips

We assembled seven wireless communications nodes into an array seen in Fig. 5a. Node 1 is the base readout node. The nodes are described in past annual reports. A 4Mhz clock and 2.5V power supply drive the arrayed nodes. The MainCounter signal trigger was wired to each breadboard node to align the masterclock edges. 610nm LEDs were connected to the inputs of the gated data and sync amplifiers. Additional 605nm LEDs were used to send data and sync light pulses. The image of the array of nodes shows the linear arrangement between nodes. The spacing between the LEDs of each node varied between 2mm and 50mm. MicroSTARLING chips are mounted in the 40 pin chip carriers.

The MicroSTARLING chip arrays will eventually work as a miniature embedded network in surgical simulators with our bioprinted corneas . For example, sufficiently small versions could track motion during surgical manipulations by monitoring the change in the network readout path. The use of optically communicating nodes makes it possible to insert and remove nodes to evaluate the timing of the formation and repair of the self-organized network. In Fig. 5a the red box identifies node 6 which is withdrawn from the network resulting in only 6 HASADATA1 readout pulses for the nodes 1, 2, 3, 4, 5 and 7. If node 6 is inserted between the nodes 5 and 7 with the LEDs aligned, a new network may be formed with all 7 nodes and 7 HasData1 pulses.

Fig. 5b shows the effect of such an insertion procedure for an input clock of 2MHz. The first readout frame in the timing diagram has 6 HasData1 pulses and node 6 is not sending data on DSS6. When node 6 is inserted between nodes 5 and 7 the second readout frame is disrupted as node 6 attempts to send data to node 5 (single pulse at 100ms). After a few milliseconds nodes 5 and 6 form a new partnership and node 6 readily receives data from node 7 (node readout at 108ms). In the third frame readout all 7 nodes are stably readout. The time to repair the network is less than 80ms, the duration between the last stable readout and the reformed readout of node 7. Fig. 5c presents network repair after node removal. In this case there are several attempts at data transmission by node 7 (124ms – 140ms) before a successful transmission to node 5. The maximum repair time in this example is 140ms. The third frame readout is stable with 6 nodes.

The real-time data shown in Figure 5 b and c is recorded with the FPGA based acquisition mini-computer and transmitted into MATLAB. This required the use of the Zybo7 device detailed in previous reports.



(a)

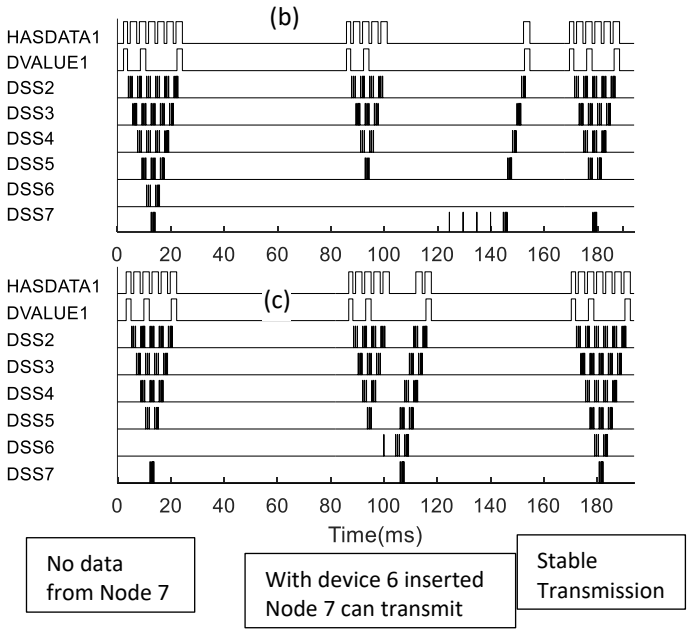


Figure 5. Sensor readout with optical communication using on chip transimpedance amplifiers with LEDs and photodiodes. The actual breadboard nodes are shown in (a) with the 6th node (red box) pulled out of the array. The photodiode sensor of node 5 (green arrow) is illuminated and results in a readout of DataValue1 = 1 in the inset timing diagram. Removal and insertion of a node results in self organized network repair in less than 180ms, representing 2 readout frames for a 2MHz input clock.

CDRL revision

CDRLs were submitted in April 2020 and reviewed by the agency. We revised the CDRLs A001 through A003 during this fourth year and are in process of resubmitting them.

Specific Aim 3

1) Major Activities (Accomplishments)

We continued to refine tissue fabrication to improve surgical "feel" based on surgeon feedback from our 2019 ocular trauma course study. We prepared for the 2021 ocular trauma course after the cancelation of the 2020 course. We integrated instrument tracking data from the Pulhemus system into MATLAB for display.

2) Specific Objectives

Our specific objectives are found in the subtasks listed below. The quarter in which they are to be completed is shown. Objectives under each task, that has been performed in year 4, are identified in the bullets. Some objectives are still in progress and thus not listed such as we currently are working to fabricate completed corneal constructs for the May 2021 ocular trauma course under Task 3.2.1.

3.1.4. Fabrication and assembly of multiple copies of the tissue holder – Q17
3.2.1. Assembly of completed device with synthetic tissue and tracking system – Q17
- Improve software for tracking system

3.2.2. GME trainee testing on three wound scenarios at Tri Service Ocular Trauma Course – Q17
- Plan 2021 Ocular Trauma Course with new leader, Dr. James Weightman.

3.2.3. Assessment of GME performance using modified OSATS scoring criteria – Q17
3.2.4. Ophthalmology Program Director survey of system utility in training – Q17
3.3.0. Delivery and revisions of CDRLs A001-A009 for all Tasks - Q17
- CDRLs need to be delivered in 2020 and revisions by 2021.

3) Significant Results from Year 4

3.1.1. Procurement and software integration of Pulhemus surgical instrument tracking system

A secondary part of the project is to track the instrument motion while the surgeon works. The Polhemus system has been assembled and used to track instrument motion. The Pulhemus tracking system (Figure 6) uses magnetic sensors to detect the change in magnetic field as small magnets are moved within range of the sensors. The sensors will be placed onto the instruments used by the surgeons and the motion tracked and linked to the tissue particle tracking data. The native software has a good display of the motion and saves the data to disk. However, it can be difficult to plot an entire procedure or link the data with other tracking data. To address these issues, we developed a function to import the Polhemus data into MATLAB. The resulting tracking data is shown in Figure 7. Figure 7 shows the traces formed during three mock suture motions. The x,y and z position is shown, however we also have information on the azimuth, elevation and rotation at each data point to shown how a surgical trainee is holding the instruments.

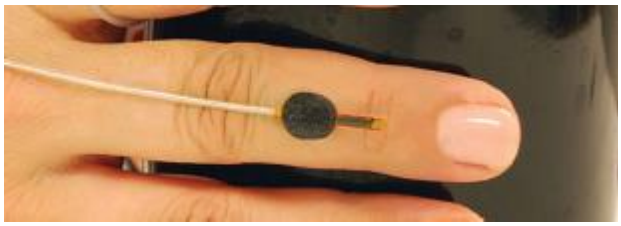


Figure 6 The Polhemus micro sensor is a 1.8 mm outer diameter magnetic sensor which is on a flexible cable. The device can be easily mounted to forceps with an adhesive or fixing pad.

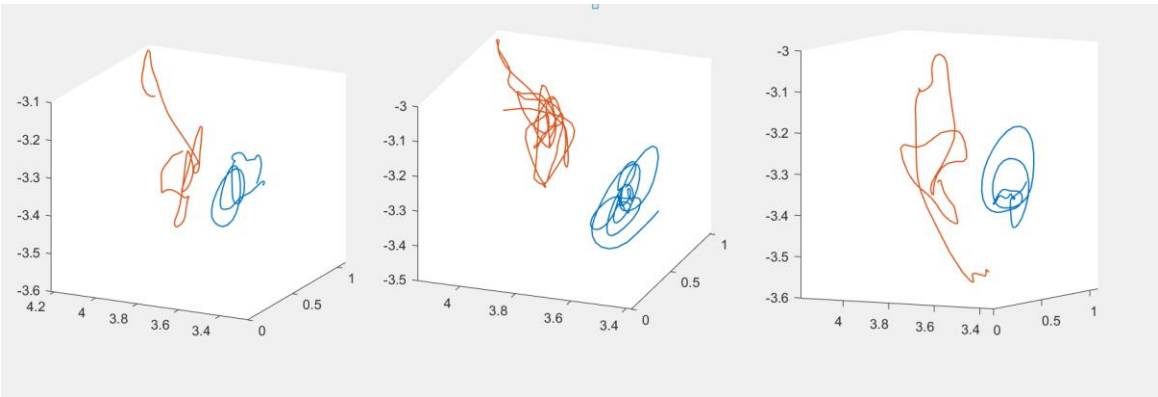


Figure 7. The track of two hands during a mock surgical suturing recording. The units on the x, and z axes are in cm showing the 100 micron resolution capability.

Discussion of stated goals not met

We have demonstrated the ability of the Microchips to communicate optically, consume less than 12 uW of power, form an array, and detect motion within the array. Due in part to the disruption of COVID we were not able to send the design out for a second fabrication run to remove the bond pads and use only the central core of the device. The central core is less than 300 microns and would have been more suitable for tracking in the cornea tissue models. However, if we are able to obtain future funding all the foundational work has been done to demonstrate the chips can function as embedded tracking units in flexible bioprinted tissue. We have prepared and submitted the chip data for publication in the IEEE Access journal. We plan to use the microspheres method of motion tracking for our May 2021 Ocular Trauma Course evaluation.

What opportunities for training and professional development has the project provided?

In past years, we presented our lab to a group of high school students as part of a tour and Uniformed Services University community outreach for STEM. In May of 2019 we were participants with our preliminary tissue construct being used for a subset of trainees and expert surgeons. This year we had less interaction due to COVID and the 2020 Ocular Trauma Course was cancelled. We are working to fabricate material for the 2021 Course and plan on mentoring a medical student now that the USU lab has reopened such interactions.

How were the results disseminated to communities of interest?

In past years, a poster was presented at MHSRS 2019 and a manuscript is detailing our humidity, voltage and solution parameters for direct write electrospinning was published in MDPI Biomaterials. In the year of concern for this report, we have submitted a paper to IEEE Access.

What do you plan to do during the next reporting period to accomplish the goals?

We will fabricate the thick layered ophthalmic constructs with our new robotic multimodal printer for use in the Ocular Trama Course in 2021. We will mainly focus on the following tasks from our statement of work listed below.

3.1.4. Fabrication and assembly of multiple copies of the tissue holder
3.2.1. Assembly of completed device with synthetic tissue and tracking system
3.2.2. GME trainee testing on three wound scenarios at Tri Service Ocular Trauma Course
3.2.3. Assessment of GME performance using modified OSATS scoring criteria
3.2.4. Ophthalmology Program Director survey of system utility in training
3.3.0. Delivery and revisions of CDRLs A001-A009 for all Tasks

4. IMPACT:

This component is used to describe ways in which the work, findings, and specific products of the project have had an impact during this reporting period. Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

- the development of the principal discipline(s) of the project;
- Other disciplines
- Technology transfer; or
- Society beyond science and technology

What was the impact on the development of the principal discipline(s) of the project?
The new director of the Walter Reed/USU Ocular trauma course (Dr Weightman) is interested in further developing the surgical simulation system for ocular and non-ocular microsurgical techniques. We are working to draft a proposal for simulation research to continue the project.

What was the impact on other disciplines?
After sharing our results with researchers interested in culturing cells in electrospun mats, we have worked to provide sample constructs for 3D culturing of primary cells for use in the discipline of heterotopic ossification in wounded warriors. The researchers are interested in studying the genes expressed in injury and recovery. In 2020 the group determined that growing cells on our structured collagen fiber constructs caused a stronger expression of FN1/GAPDH than their standard monolayer cell cultures

What was the impact on technology transfer?
We have obtained a patent for the microchips as embedded array motion tracking units. The patent (10,819,437) was issued in October 2020.

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

5. CHANGES/PROBLEMS:
The Project Director/Principal Investigator (PD/PI) is reminded that the contract organization is required to obtain prior written approval from the awarding agency Contracts Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

- Changes in approach and reasons for change.
- Actual or anticipated problems or delays and actions or plans to resolve them.
- Changes that have a significant impact on expenditures.
- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Changes in approach and reasons for change
No changes in approach have occurred during this phase of the project.

Actual or anticipated problems or delays and actions or plans to resolve them
No delays actual or anticipated.

Changes that had a significant impact on expenditures
COVID affected our expenditures in that we adjusted the rate of expenditure to allow for participating tin the 2021 Ocular Trauma Course.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
No changes have occurred this reporting period.

6. PRODUCTS:
List any products resulting from the project during the reporting period. Examples of products include:

- publications, conference papers, and presentations;
- website(s) or other Internet site(s);
- technologies or techniques;
- inventions, patent applications, and/or licenses; and
- other products.

Publications, conference papers, and presentations
"Nothing to Report."

Journal publications.
Alexander, F. A., Johnson, L., Williams, K. & Packer, K.; A Parameter Study for 3D-Printing Organized Nanofibrous Collagen Scaffolds Using Direct-Write Electrospinning. ;

Materials (Basel).; 2019; 12, 4131 Published; acknowledgement of federal support (yes).

Statement "This research was funded by US Army Medical Research and Materiel Command (USAMRMC), award number W81XWH-17-C-003. The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation."

Books or other non-periodical, one-time publications.
"Nothing to Report."

Other publications, conference papers, and presentations.
MHSRS poster presentation 2019.

Website(s) or other Internet site(s)
"Nothing to report"

Technologies or techniques
New techniques for high speed, closed loop electrospinning and microsensor communication. Consideration of the patentability of the methods developed by subcontractor Meadowave is pending successful demonstration of system. Once patentability is determined disclosure will be made to USAMRMC and patents applied for. The methods will then be published in an appropriate journal.

Inventions, patent applications, and/or licenses
Patent application in process for MINIATURE EMBEDDED SELF-ORGANIZED OPTICAL NETWORK. Patent has been disclosed to the government using form DD882. If awarded, patent will indicate government usage rights.

Other Products
We have produced demonstration ophthalmic constructs that will be used in the USUHS Ocular Trauma Course in May of 2019 and should lead to training benefits by year 3 of the project when the full system is complete. The techniques and feedback from trainees will be reported to USAMRMC as part of our CDRLs. Also, we have produced a circuit diagram for the sensor prototype chips that will lead to training benefits by year 3 and be reported to USAMRMC as part of our CDRLs.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?
Name: Kyle Packer
Project Role: Principal Investigator
Researcher Identifier: N/A
Nearest Person Month Worked: 2
Contribution to Project: Dr. Packer contributed in the areas listed below. He managed project personnel tasks and reporting. He guided purchasing choices and procurement schedule, laboratory space search and setup. He oriented new project personnel and directed work strategies of project personnel. He assured compliance with project requirements.
Funding Support: Ophthalmologist at WOMC Fort Bragg, NC

Name: Lee Johnson
Project Role: Co-I
Researcher Identifier: N/A
Nearest Person Month Worked: 12
Contribution to Project: Dr. Johnson completed or initiated tasks related to FPGA system design, electrospinning system design, equipment and materials procurement, software procurement and installation, software coding in VHDL, selection of microparticles for deposition and selection of 3D bioprinter. Dr. Johnson also defined laboratory space requirement and oriented new project personnel. He directed the daily tasks of the project personnel.
Funding Support: N/A

Name: Frank Alexander
Project Role: Postdoctoral Researcher
Researcher Identifier: N/A
Nearest Person Month Worked: 12

Contribution to Project: Dr. Alexander completed or initiated the electrospinning system assembly, software coding in LabView, performance of data collection and analysis for microsphere penetrations and electrospinning.
Funding Support: N/A

Name: Bryan Stevens
Project Role: Medical Student laboratory rotation
Researcher Identifier: N/A
Nearest Person Month Worked: 2
Contribution to Project: Bryan Stevens assisted Dr Alexander in preparation of the Cellink printer, determining viscosity measurement methods, preparing collagen solutions and testing the electrospinning printer.
Funding Support: N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period
"Nothing to Report."

What other organizations were involved as partners?
Organization Name: Naval Research Laboratory, Chemistry Division
Location of Organization: 4555 Overlook Avenue, Washington, DC 20375
Partner's contribution to the project: Collaboration with Dr. Russell Kirk Pirlo

Organization Name: University of Florida, Department of Electrical Engineering
Location of Organization: University of Florida, Gainesville, FL 32611
Partner's contribution to the project: Collaboration with Dr. William Eisenstadt

8. SPECIAL REPORTING REQUIREMENTS:
QUAD CHARTS: The Quad Chart (available on https://www.usamraa.army.mil) shall be updated and submitted as an appendix.

9. APPENDICES:
- Quad Chart

Development of 3D printed Ophthalmic Tissue for Surgical Training

Log Number: BA150090

Contract Number: W81XWH-17-C-0003

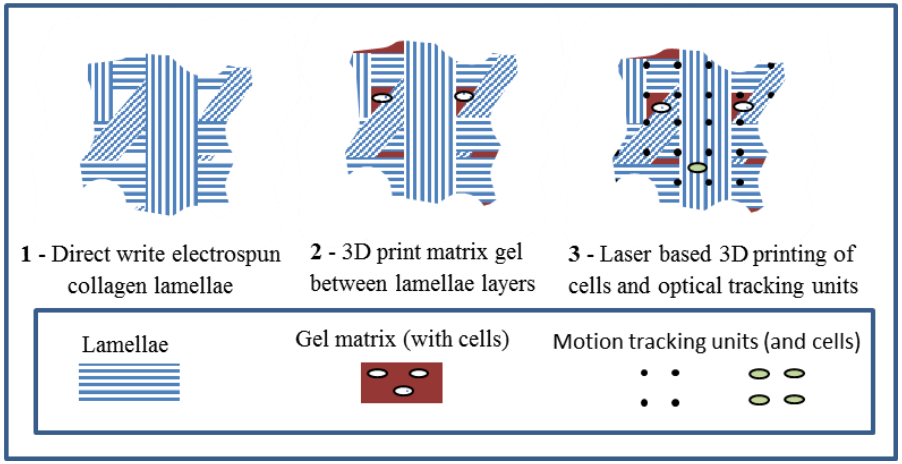
PI: CPT Kyle Packer, MD **Org:** The Geneva Foundation **Award Amount:** 2,165,174

Study/Product Aim(s)

1. Successfully utilize 3D bioprinting technologies to create a critical component of a cost-effective and realistic simulated tissue corneal and scleral wound repair simulator system.
2. Successfully design, fabricate and 3D print microscale tracking units to a surgical motion and intrinsic tissue response to manipulation recording component as an integral part of the surgical simulation system.
3. Successful integration of 3D bioprinted scleral and corneal tissue with intrinsic tissue motion tracking to a pressurized surgical training system used to standardize GME surgical training modules.

Approach

1. Unlike most groups, we plan to use multiple modes of 3D printing. Novel direct write electrospinning will be used to approximate the ultrastructure of the cornea with nanoscale collagen fibrils woven to form microscale lamellae. Laser induced forward transfer will be used to deposit fibroblasts and keratocytes. Gel extrusion printing will hold layers together.
2. Two methods of optical readout of position changes will be developed in parallel: active wireless readout of optoelectronic microchips and passive imaging of fluorescent micro-spheres for risk mitigation.



Accomplishments: Design of electrospinning and optical tracking unit development systems. Procurement of system materials and equipment. Transfer of existing Matlab software code to preliminary VHDL FPGA code for tracking units.

Timeline and Cost

Activities	CY 17	18	19	20
Specific Aim 1 – create realistic tissue corneal and scleral wound repair system				
Specific Aim 2 – creation of 3D printed microscale tracking units				
Specific Aim 3 - integration of 3D bioprinted scleral and corneal tissue with intrinsic tissue motion tracking				
Estimated Budget ($K)	**$842**	**$673**	**$618**	

Updated: 20 March 2021

Goals/Milestones

CY17 Goal – Acquisition, Assembly and Testing of Systems

✓Electrospinning and positioning system

✓Version 1.0 Hardware code for FPGA based tracking system

CY18 Goals – Validation of electrospinning and tracking subsystems

✓3D collagen lamellae electrospinning with crosslinking

✓Fabrication of ASIC tracking microchips

CY19 Goal – System Refinement

✓Demonstration of 3D printed synthetic scleral and corneal tissue

✓Laser printing of cells and arrays of functional tracking units

CY20 Goal – System integration and trainee testing

☐ GME trainee testing with 3 wound types using completed system

Comments/Challenges/Issues/Concerns

None to report.

Budget Expenditure to Date

Projected Expenditure to Date: $2.1M

Actual Expenditure: $2.1M