AWARD NUMBER: W81XWH-17-C-0003

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

PRINCIPAL INVESTIGATOR: Kyle Packer, MD

CONTRACTING ORGANIZATION: The Geneva Foundation

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13. SUPPLEMENTARY NOTES

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.

15. SUBJECT TERMS

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Table of Contents

Page

1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

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: Provide a brief list of keywords (limit to 20 words).

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 percentage of completion and target dates are shown for each task of the project. The actual completion quarters of subtasks are also shown for subtasks 90% or more completed. There have no been significant changes in approach or methods from the agency approved application or plan. The addition of 4 months to the project under the approved no cost extension has resulted extensions of the quarters in which some tasks were to be completed. These are generally noted as Quarter 13 and Quarter 14.

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 - Q2

1.1.2. Electrospin Collagen fibers of nano- and microscale size – Q3 — 100% complete/Target Q8

1.1.3. Electrospin individual fibers into lamellae with or without nanopositioner orientation and determine Young's Modulus – Q5

1.1.4. Electrospin collagen fibrils with Adept robot 3D positioning to form ophthalmic constructs and determine Young's Modulus – Q8

1.2. Direct- write 3D bioprinting of Gel MA and crosslinking compounds.	100% Complete / Target Q8
1.2.1. Acquire and commission 3D bioprinter – Q3	
1.2.2. Demonstrate 3D deposition of hydrogels onto electrospun collagen – Q4	
1.2.3. Demonstrate crosslinking of electrospun collagen and 3D bioprinted hydrogels and determine Young's Modulus – Q7	
1.3. 3D printing of living cells	100% Complete / Target Q10
1.3.1. Deposit living cells with BioLP or 3D BioLP/LIFT bioprinter onto culture dish – Q7	
1.3.2. Deposit living cells into Gel MA gel matrix – Q8	
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	100% Complete / Target Q12
2.1.1. Design, assemble and evaluate FPGA based circuits for wireless nodal communication – Q4	
2.1.2. Transfer FPGA design to ASIC microchips with bonded LEDs and photodiodes – Q6	
2.2. Precision 3D placement of microscale tracking units using BioLP based method	100% Complete / Target Q10
2.2.1. Deposition of 20, 40 and 100 micron microspheres and microchips into gel structures – Q2	
2.2.2. Quantification of depth of penetration into gel structures and accuracy of placement – Q3	
2.3. Development of wireless microchip tracking system	80% Complete / Target Q12
2.4. Development of an optically based microsphere tracking system	100% Complete/Target Q9
2.4.1. Design and acquisition of camera based optical particle tracking system and software – Q7	
2.4.2. Demonstration of particle location and tracking on dry surface – Q7	
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	50% Complete / Target Q13
3.2. Surgical evaluation and collection of data for standardized nomogram.	50% Complete / Target Q13
3.3. Delivery and revisions of CDRLs A001-A009 for all Tasks	50% Complete / Target Q14

What was accomplished under these goals?

For this reporting period describe:
Specific Aim 1
1) major activities (accomplishments);

In this second year of performance our major accomplishments include: forming thin ophthalmic constructs with electrospun collagen fibrils and crosslinked gel matrix; determining Young's moduli for the human cornea, plain collagen fibrils, and combined fibrils and gel matrix; depositing living cells onto the ophthalmic constructs with our prototype bio-laser printer and constructing a robot-based printer which allows for fast switching between electrospinning and gel extrusion print modes.

2) specific objectives;

Our specific objectives are found in the subtasks listed below. The quarter in which they were completed is identified after the task title. Quarters in **bold font** are new this year and detailed in the significant results section.
1.1.1. Assemble electrospun apparatus with 3D positioning - Q2
1.1.2. Electrospin Collagen fibers of nano- and microscale size – Q3
1.1.3. Electrospin individual fibers into lamellae with or without nanopositioner orientation and determine YM – Q5
1.1.4. Electrospin collagen fibrils with Adept robot 3D positioning and determine Young's Modulus – Q8
1.2.1. Acquire and commission 3D bioprinter – Q3
1.2.2. Demonstrate 3D deposition of hydrogels onto electrospun collagen – Q4
1.2.3. Demonstrate crosslinking of electrospun collagen and 3D bioprinted hydrogels and determine YM – Q7
1.3.1. Deposit living cells with BioLP or 3D BioLP/LIFT bioprinter onto culture dish – Q7
1.3.2. Deposit living cells into Gel MA gel matrix – Q8
1.3.3. Deposit living cells into 3D formed ophthalmic constructs – **Q10**

3) significant results

Significant results for quarters 1-4 have already been described in the Year 1 Annual Report. Below are significant results from project year 2.

Deposit living cells into 3D formed ophthalmic constructs

Detailed Description:

We have deposited living cells onto our constructs. The constructs are made of 3D printed collagen fibers on a 3D printed Gel mat. The goal of this step is to incorporate cells into the constructs to make them more "life-like" for the surgeon. As the cells in the cornea are sparse, the cells will not impede the scalpel or needle during injury creation and suturing but may have some impact on the collagen via remodeling mechanisms. We placed Hela cells and hNFF cells onto the constructs. The cells show good viability after two days. They are incorporated into the fibers upon placement at day 0 but appear follow the fiber structure after one or two days of incubation.

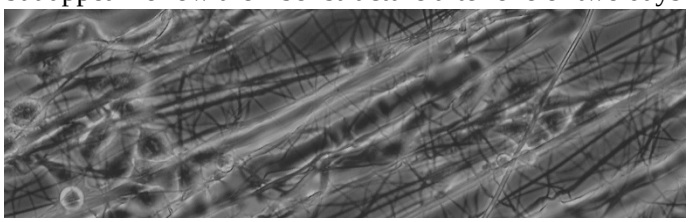


Figure 1a. Day 0 HeLa cells on a collagen fiber corneal construct. The Fibers can be seen with some cells identified with arrows.

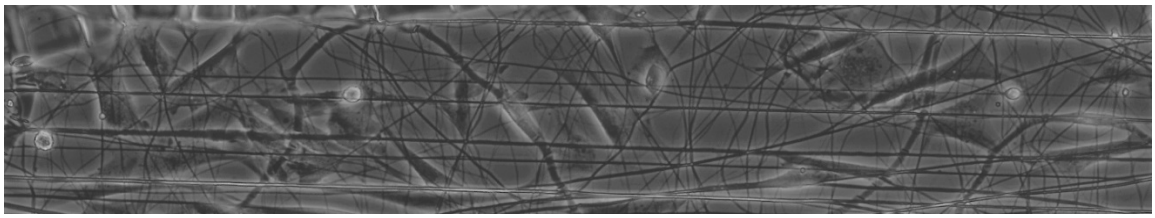


Figure 1b. Day 0 hNFF cells seen on the collagen fiber corneal construct. Some cells are interacting with the fibers.

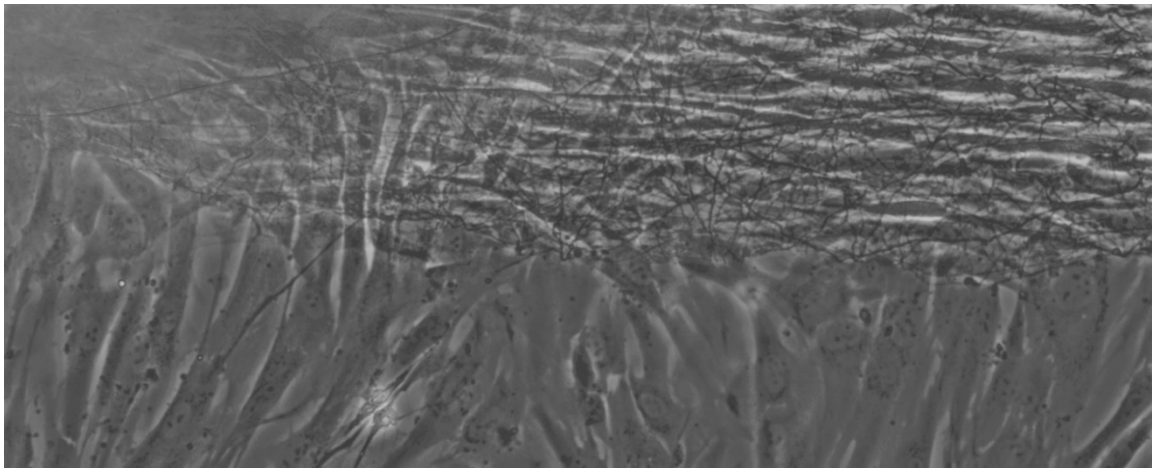


Figure 1c. Day 2 hNFF cells have proliferated and the orientation clearly follows the region of horizontal 3D printed collagen fibers. Cells are from a different region than previous image.

Specific Aim 2

1) major activities (accomplishments);

Specific Aim 2 is to 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. We obtained a major milestone this year of transferring our FPGA based design into a circuit that can be microfabricated with the acquired Mentor Tanner electronic design automation software. We also demonstrated optical particle tracking of microspheres with a modified motion capture system.

2) specific objectives;

Our specific objectives are found in the subtasks listed below. The quarter in which they were completed is identified after the task title. Quarters in bold font are new this year and detailed in the significant results section.

2.1.1. Design, assemble and evaluate FPGA based circuits for wireless nodal communication – Q4
2.1.2. Transfer FPGA design to ASIC microchips with bonded LEDs and photodiodes – Q6
2.1.3. Evaluate microchip array read out and modify design as needed – **Q9**
2.1.4. Fabricate custom microchips with incorporated optoelectronics using MOSIS institute -**Q13**
2.2.1. Deposition of 20, 40 and 100 micron microspheres and microchips into gel structures – Q2
2.2.2. Quantification of depth of penetration into gel structures and accuracy of placement – Q3
2.2.3. Quantification of deposition into collagen/gel ophthalmic constructs – **Q9**
2.2.4. Fabrication of BioLP printer head at Walter Reed – **Q10**
2.3.1. Design control unit for wireless readout of positional data from nodal sensor array – **Q10**
2.3.2. Fabricate FPGA based control unit and with software for readout and position tracking – **Q12**
2.3.3. Readout data from microchip array imbedded in gel matrix to user interface - **Q12**
2.4.1. Design and acquisition of camera based optical particle tracking system and software – Q7
2.4.2. Demonstration of particle location and tracking on dry surface – Q7
2.4.3. Demonstrate particle location and tracking in gel matrix – **Q9**

3) Significant Results

Significant results for quarters 1-8 have already been described in the Years 1 and 2 Annual Report. Below are significant results from project year 3.

2.1.3. Evaluate microchip array read out and modify design as needed – Q9
2.1.4. Fabricate custom microchips with incorporated optoelectronics using MOSIS institute -Q13

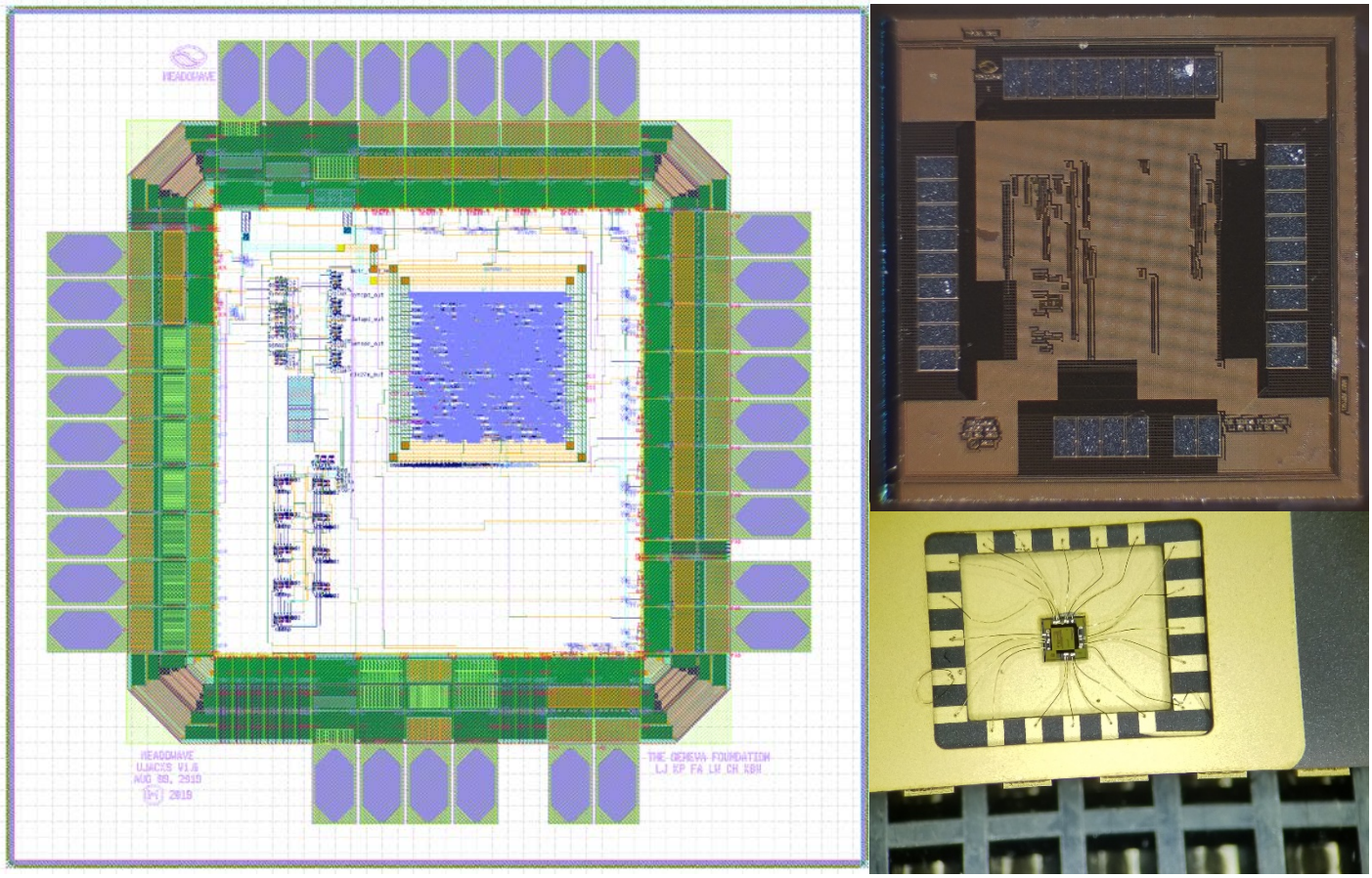


Figure 2. The imagemap of the chip layout on the left and the fabricated chip on the right. The chip area is approximately 1250 x 1250 microns.

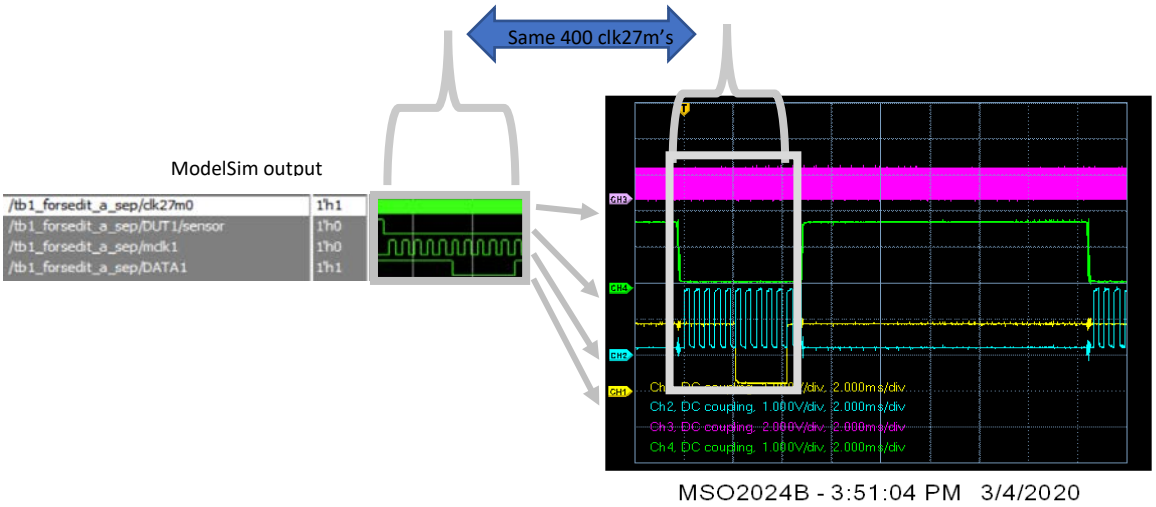


Figure 3. The actual output from the chip. The Left side shows the output of the software simulation of the chip. The right side shows the lines or traces are from the oscilloscope connected to the actual chip. The 4 gray arrows show how the simulation voltage traces match the actual measured traces. This demonstrate the successful function of the chip. But not chip to chip communication.

2.2.3. Quantification of deposition into collagen/gel ophthalmic constructs – Q9

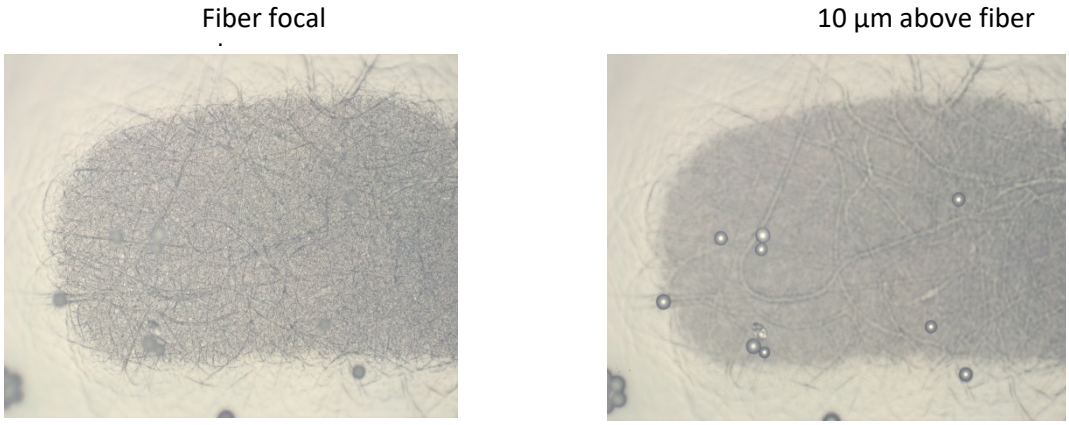


Figure 4. The deposition of microspheres into collagen/gel ophthalmic constructs.

This goal for this task was to use a BioLP to deposit microspheres into our gel/fiber constructs. Figure 4 shows the beads resting on the surface of the gel fiber constructs. This result is different than the result we obtained with gel only constructs reported in our Year 1 Annual report. Previously the microspheres readily penetrated the gel. Here the microspheres do not penetrate the fiber regions only the surface gel. We can use the method of BioLP printing of microspheres but not more than 10 to 20 microns of depth.

2.2.4 Fabrication of BioLP printer head at Walter Reed – Q10

This task was completed in a previous quarter as a prototype instrument. We have improved the system by adding a proper femtosecond laser operating at 100KHz with 800 femtosecond pulses. The pulse energy was tested in our lab at 25 microjoules (uJ). The literature shows that the pulse energy range to create drops of bioink goes from 21 to 33 uJ for ns pulses to 3 to 3.5 for femtosecond pulses. In addition, it is possible with femtosecond pulses to project drops of bioink without a gold absorbing layer. After testing the function and power output of the Laser-Femto Uranus laser, we incorporated it into our existing BioLP setup, Figure 5. We were able to create marks in the gold absorbing layer and focus the laser spot size to approximately 100 microns.

Figure 5. Image of the on-site BioLP. We have assembled a prototype biolaser printer at our facility. We used the printer to deposit cells into the gel constructs printed with our extrusion (Cellink) bioprinter.

2.3.1. Design control unit for wireless readout of positional data from nodal sensor array – Q10

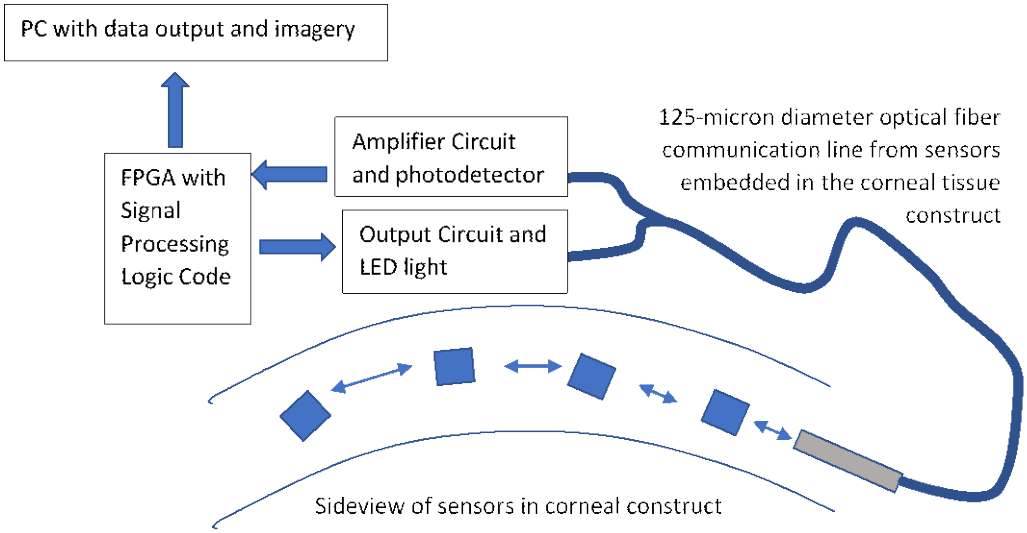


Figure 6. The readout system design.

The goal of this task is to assemble a software and hardware system to readout from the networked chips implanted in the corneal construct. Fig. 6 shows the system design. There are three parts to the system: the optical transducer, the communications processor and the data display software. The optical transducer involves a LED photodetectors connected to light collection devices (such as fiber optic lines) and analog amplifiers. The communications processor is the same as the processor running on the fabricated chips. It could be the digital portion of one of the chips or it could be a FPGA running similar program as is hardwired onto the chips. The FPGA gives the flexibility to adjust the code and interface to more powerful analog amplifiers and LEDs than exist on the low power chips. The data display software will take the readout of the array and display the relative position of the array chips. To enable the development of the FPGA based control system separate from the design, fabrication and testing of the actual microchips, we have used the printed circuit board versions of the chips shown in Fig. 7C. These boards have the analog amplifier circuit, a 12 MHz clock and a 2mm FPGA commercial chip running our communications processor code. These will be replaced first with a group of chips wire bonded together (Fig. 7B) and eventually with the wireless form of the chips, i.e. the same chips with the optical components required for wireless communication. Figure 7A shows the PC software interface (VIVADO by Xilinx Inc.) and the mini-computer board with built in FPGA (Zybo Z7). The FPGA part of the Zybo Z7 device is loaded with the communications processor code. The ARM CPU of the Zybo Z7 can run the interface software and display the data on a screen. Thus, the Zybo Z7 houses the communications processor and data display software and the amplifiers and LED/fiber optics will function as the optical interface. The Zybo FPGA processor will also output the master clock sync that is required to sync the timing of the chips.

2.3.2. Fabricate FPGA based control unit and with software for readout and position tracking – Q12

We assembled the FPGA based microchip control unit according to the design of Task 2.3.1. Figure 7 shows the system components.

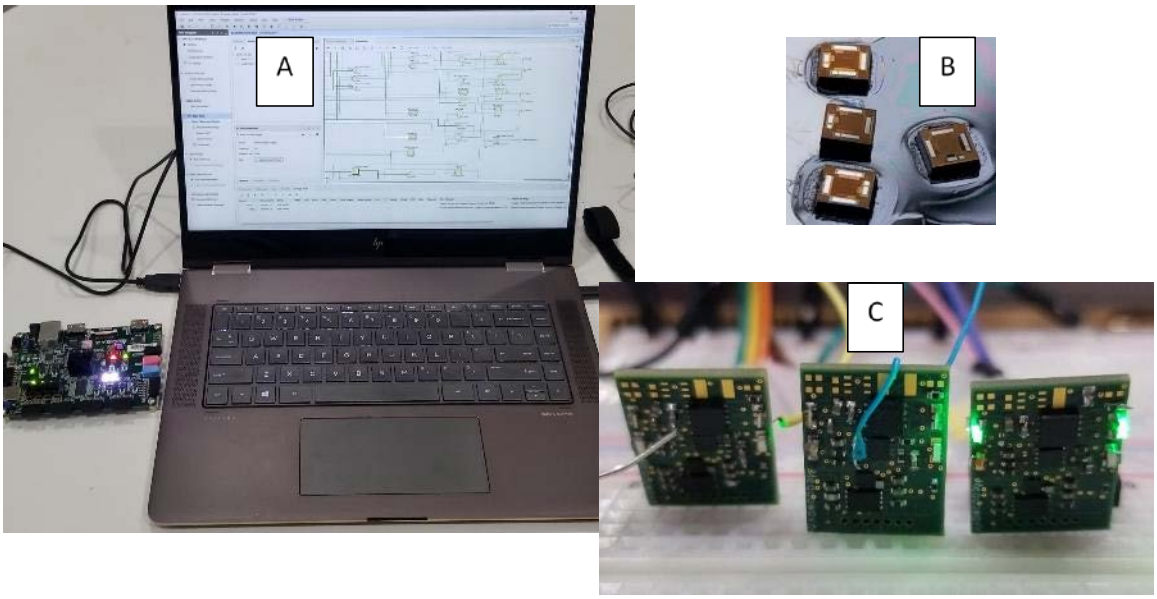


Figure 7. The prototype chip array readout system. The Zybo FPGA and minicomputer board is shown in A. The array of un-wire bonded microchips which will be used to refine the system are in B. The printed circuit board versions of the wireless devices which are used to create the first version of the system are shown in C.

2.3.3. Readout data from microchip array imbedded in gel matrix to user interface - Q12

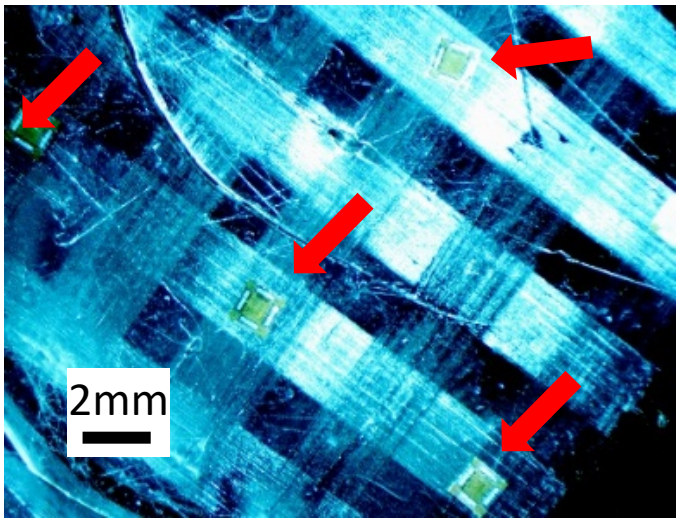


Figure 8. Image of the collagen fibers (crosshatch pattern) embedded into the gel. The chips (red arrows) were spaced about 8mm apart.

The goal of this task is to deposit microchips into the collagen fiber/ gel constructs and readout from the array of chips. We have placed the chips into the constructs as seen in Figure 8. We are currently testing the communications feature of the chips but have shown the proper function of the digital circuits (Figure 3).

Specific Aim 3
1) Major Activities (Accomplishments);

Specific Aim 2 is to 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. We obtained a major milestone this year of transferring our FPGA based design into a circuit that can be microfabricated with the acquired Mentor Tanner electronic design automation software. We also demonstrated optical particle tracking of microspheres with a modified motion capture system.

2) Specific Objectives;

Our specific objectives are found in the subtasks listed below. The quarter in which they were completed is identified after the task title. Quarters in bold font are new this year and detailed in the significant results section.

3.1.1. Procurement and software integration of Pulhemus surgical instrument tracking system – **Q10**
3.1.2. Fabrication of tissue holder with integrated tracking electronics - **Q11**
3.1.3. Completion of software for surgeon user interface and functional testing – **Q12**
3.1.4. Fabrication and assembly of multiple copies of the tissue holder – Q13
3.2.1. Assembly of completed device with synthetic tissue and tracking system – Q13
3.2.2. GME trainee testing on three wound scenarios at Tri Service Ocular Trauma Course – Q13
3.2.3. Assessment of GME performance using modified OSATS scoring criteria – Q13
3.2.4. Ophthalmology Program Director survey of system utility in training – Q14
3.3.0. Delivery and revisions of CDRLs A001-A009 for all Tasks - Q14

3) Significant Results

3.1.1. Procurement and software integration of Pulhemus surgical instrument tracking system – Q10

The Polhemus system has been requested and the software will be delivered with the system. The picture below shows the system and microsensors which will be attached to the surgical forceps to be used.

Figure 9a. The Polhemus data acquisition unit (right) and magnetic source (left)

Figure 9b. The 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.

The Pulhemus tracking system (Figure 9a) uses magnetic sensors (Figure 9b) 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.

3.1.2. Fabrication of tissue holder with integrated tracking electronics - Q11

The tissue holder in use with electronics for motion capture integrated. The final chip tracking electronics will be added if the current generation chip tracking system is determined to function as an improvement on the particle tracking.

Figure 10. The tissue holder with the tracking system electronics integrated as a "patient head". The surgeon (B) sutures the corneal construct placed within the tissue holder (A). The tissue holder is placed into the gray box (arrow) which represents the patient head. The gray box houses the electronics shown in C.

3.1.3. Completion of software for surgeon user interface and functional testing – Q12

The user interface for the particle tracking system was custom designed to replace the software that comes with the particle tracking system. The key challenges were tracking 100um particles and simplifying the interface for novice users. The Optitrack system is used to perform motion tracking for video game and CGI movie animations. Two or more cameras image retroreflective objects of 1 to 3 cm size. We are using particles of 30 to 100 microns which are harder to illuminate and image. We needed to change the optics from distance focusing camera lenses to microscope objectives. We also had to simplify the user interface. After our trial demonstration at the 2019 Ocular trauma Course, we realized the user interface was overly complex (Figure 11) and did not clearly show the surgeons how the tissue was responding to manipulation. We wrote a custom program in C++ to operate the cameras.

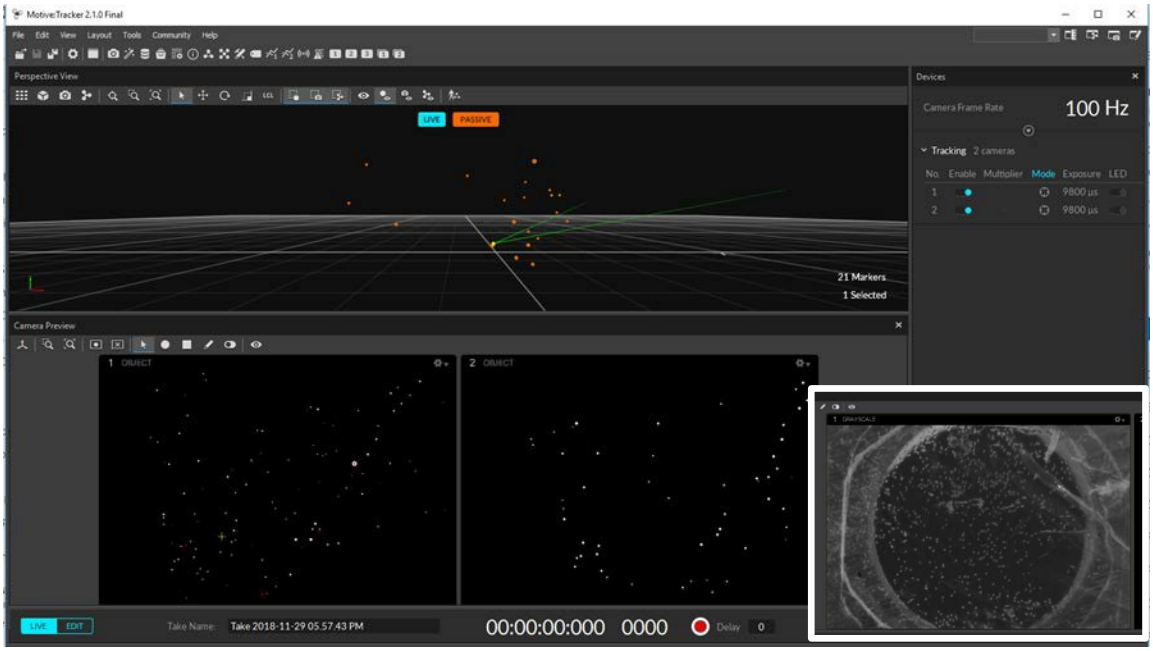


Figure 11. Screen capture of original complex software with a difficult to understand interface.

3.1.4. Fabrication and assembly of multiple copies of the tissue holder – Q13

We fabricated multiple copies of the 3D printed corneal tissue construct holders to allow rapid insertion into the tracking system during the Ocular Trauma Course.

Figure 12. Image of a collection of the tissue holders.

Discussion of stated goals not met

We have placed the fabricated microchips into the fiber/gel constructs and we have tested the chip function and found it acceptable for communication. We have not yet demonstrated communication within the gel/fiber construct. We will attempt this in the next quarter. We plan to use the microspheres method of motion tracking for our May 2020 Ocular Trauma Course evaluation.

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

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

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.

How were the results disseminated to communities of interest?

A poster was presented at MHSRS 2019. A manuscript is detailing our humidity, voltage and solution parameters for direct write electrospinning was published in MDPI Biomaterials.

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

In general terms, we will fabricate the sensor chips, produce thick layered ophthalmic constructs with out new robotic multimodal printer and develop the hardware and software for use in the Ocular Trama Course in 2020. 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 – Q13
3.2.1. Assembly of completed device with synthetic tissue and tracking system – Q13
3.2.2. GME trainee testing on three wound scenarios at Tri Service Ocular Trauma Course – Q13
3.2.3. Assessment of GME performance using modified OSATS scoring criteria – Q13
3.2.4. Ophthalmology Program Director survey of system utility in training – Q14
3.3.0. Delivery and revisions of CDRLs A001-A009 for all Tasks - Q14

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?

Our presentation at MHSRS 2019 was well received and has lead to interest in future collaborations. Our paper has lead to contacts from labs at Wake Forest University interested in replicated our procedures to direct write electrospin.

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.

What was the impact on technology transfer?

We have applied for a SBIR to develop the concept into a 3D printing method for rapid evaluation of nanosensors.

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

Lower cost bioprinter was procured and expenditures allocated to supporting electronics for bioprinter.

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.

If there is nothing to reporder a particular item, state "Nothing to Report."

• Publications, conference papers, and presentations

Report only the major publication(s) resulting from the work under this award. There is no restriction on the number. However, agencies are interested in only those publications that most reflect the work under this award in the following categories:

Journal publications. List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like. A publication in the proceedings of a one-time conference, not part of a series, should be reported under "Books or other non-periodical, one-time publications."

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

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. Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Other publications, conference papers, and presentations. Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

MHSRS poster presentation 2019.

• Website(s) or other Internet site(s)

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

• Technologies or techniques

Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

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**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

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**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

• data or databases;
• biospecimen collections;
• audio or video products;
• software;
• models;
• educational aids or curricula;
• instruments or equipment;
• research material (e.g., Germplasm; cell lines, DNA probes, animal models);
• clinical interventions;
• new business creation; and
• other.

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

Provide the following information on participants:

• what individuals have worked on the project?
• has there been a change in the other active support of the PD/PI(s) or senior/key personnel since the last reporting period?
• what other organizations have been involved as partners?

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).

• Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.

Describe how this person contributed to the project and with what funding support. If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Example:

Name:	Mary Smith
Project Role:	Graduate Student
Researcher Identifier (e.g., ORCID ID):	1234567
Nearest person month worked:	5
Contribution to Project:	Ms. Smith has performed work in the area of combined error-control and constrained coding
Funding Support:	The XYZ Foundation (Complete only if the funding support is provided from other than this award.)

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?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active contract has closed and/or if a previously pending contract is now active. Annotate this information so it is clear what has changed from the previous submission.

Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

“Nothing to Report.”

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed. Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner's contribution to the project (identify one or more)

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
- Other.

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: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Development of 3D printed Ophthalmic Tissue for Surgical Training

Log Number: BA150090

Contract Number: W81XWH-17-C-0003

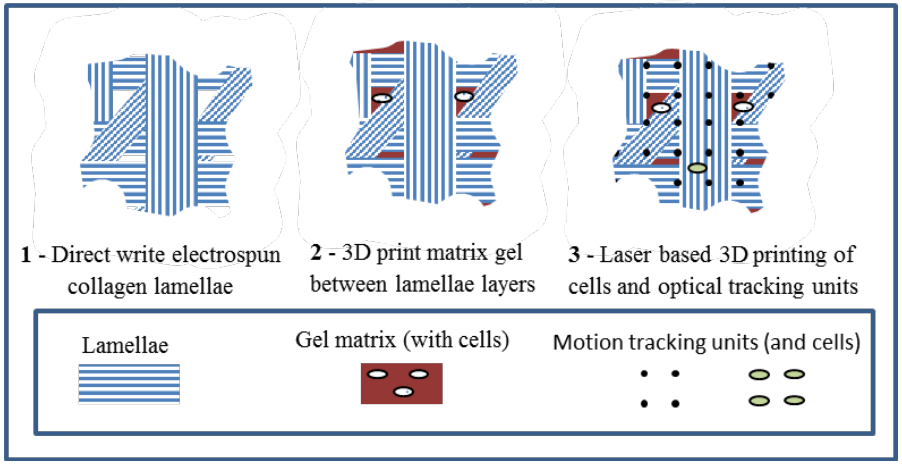
PI: CPT Kyle Packer, MD **Org:** The Geneva Foundation **Award Amount:** 2,165,174

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 2019

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: $1,515,000

Actual Expenditure: $1,295,586