AWARD NUMBER: W81XWH-17-C-0003

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

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CONTRACTING ORGANIZATION: The Geneva Foundation
Tacoma, WA 98402

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Fort Detrick, Maryland 21702-5012

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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S) Dr. Kyle Packer, MD; Dr. Lee Johnson, Ph.D.

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. **PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
The Geneva Foundation
917 Pacific Ave, Suite 600
Tacoma, WA 98402

8. PERFORMING ORGANIZATION REPORT NUMBER

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US. Army Medical Research and Materiel Command
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

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. 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.

15. SUBJECT TERMS

None listed

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

Page

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:

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 dates of subtasks are also shown for fully completed subtasks. There have been no 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. 40% Complete / Target Q8

100% Completed Subtasks with Quarter Finished

1.1.1. Assemble electrospun apparatus with 3D positioning – Q2

1.1.2. Electrospin Collagen fibers of nano- and microscale size – Q3

1.2. Direct- write 3D bioprinting of Gel MA and crosslinking compounds. 30% Complete / Target Q8

100% Completed Subtasks

1.2.1. Acquire and commission 3D bioprinter – Q3

1.2.2. Demonstrate 3D deposition of hydrogels onto electrospun collagen – Q4

1.3. 3D printing of living cells 0% Complete / Target Q10

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 25% Complete / Target Q12

100% Completed Subtasks

2.1.1. Design, assemble and evaluate FPGA based circuits – Q4

2.2. Precision 3D placement of microscale tracking units using BioLP based method 33% Complete / Target Q11

100% Completed Subtasks

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 0% Complete / Target Q9
2.4. Development of an optically based microsphere tracking system 10% Complete / Target Q9

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 0% Complete / Target Q12
3.2. Surgical evaluation and collection of data for standardized nomogram. 0% Complete / Target Q12
3.3. Delivery and revisions of CDRLs A001-A009 for all tasks 0% Complete / Target Q12

What was accomplished under these goals?

For this reporting period describe:

Specific Aim 1
1) major activities (accomplishments);

In this first year of performance we met all the goals set in the statement of work for Specific Aim 1. The most significant accomplishment was electrospinning a single layer of collagen lamellae like structures. These structures are the building block of the full cornea and scleral constructs.

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.

1.1.1 Assemble electrospun apparatus with 3D positioning – Q2
1.1.2 Electrospin Collagen fibers of nano- and microscale size – Q3
1.2.1 Acquire and commission 3D bioprinter – Q3
1.2.2 Demonstrate 3D deposition of hydrogels onto electrospun collagen – Q4

3) significant results

1.1.1 Assemble electrospun apparatus with 3D positioning

We assembled two electrospinning systems. The first, referred to as the development system, is housed in a temperature and humidity control chamber. The development system will be used to define the environmental and solution parameters of lower voltage near field electrospinning. The second system is the research system which will be used to evaluate various methods of increasing the electrospinning speed. This is important for producing sufficient ophthalmic tissue for practical use. The system components are described as follows.

The electrospinning system is comprised of a syringe pump (NE-1000X, New Era Pump Systems Inc), a high voltage source (PS350, Stanford Research Systems Inc), X and Y linear motor stages (DDSMP1/M, Thorlabs Inc) and a Z stage (MTS50A-Z8, Thorlabs, Inc) all controlled via a Labview hardware and software system (NI PXIe-1071, LabView 2017, National Instruments Inc). A high speed camera (A1300, Basler Inc) controlled via National Instruments NI-Max allowed for imaging of the syringe tip and spun fiber. We have designed a software architecture and created a first version that can control the electrospinning of the polymer and collagen fibers with 3D CAD standard inputs. A typical 3D printer uses a text file with a list of moves between specific points at given velocities and print parameters. This list is termed G-code. The use of G-code by our software means we will be able to take a standard CAD file for almost any properly sized object and print it with our electrospinning printer. A screen shot below shows the control panel for the electrospinning printer. The printer has also been moved into a humidity and temperature controlled chamber (ETS Inc, Model 5532). This will allow us to optimize the environmental conditions to print collagen with minimal needle clogging and for approximately 1mm spinning distances.

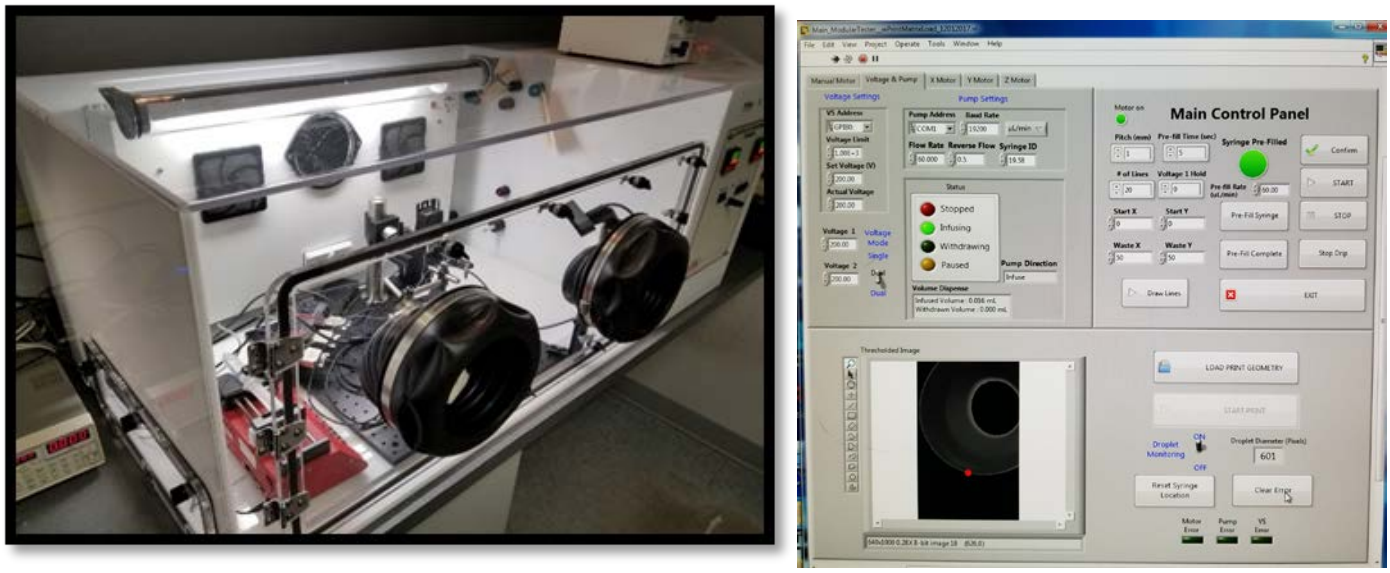


Figure 1

We first electrospun Poly Ethelene Oxide (PEO) as it is an easier material to work with as we refined our system. We used our direct write system to rapidly write 400 micron lines of polymer repeatedly in the y direction. While writing these lines the needle was slowly moved in the x direction. Our fastest line speed used was slightly over 100, 400 micron lines drawn per second. The below figure shows nanoscale (~400nm) thick oriented fibers spun with our system. Faster drawn lines are straighter.

Figure 2

Microsopy images of PEO 400 micron long nano/microfibers placed with our direct write electrospin system. Approximately 100 lines per second were drawn in the leftmost image and 40 lines per second in the center image. The rightmost image shows an SEM of a 400nm fiber.

1.1.2 Electrospin Collagen fibers of nano- and microscale size – Q3

After obtaining preliminary data showing we can direct write PEO fibers, we used Calfskin derived collagen dissolved in HFIP as our spinning solution. We varied the concentration of collagen in solution to begin to optimize the spin parameters. We found that for electrospinning with 700 to 1200V applied to the needle relative to the gold substrate 8% w/w collagen in HFIP could be electrospun at approximately 1mm distance from the surface. The fibers leave the Taylor cone of the solution drop at a rate dependent in part on the voltage applied. Lines drawn at slightly over 100Hz had straighter profiles versus lines drawn at slightly less than 40Hz, which looped and curled as the electrospinning process produced fibers faster than the needle was moved. When the y axis is shifted to draw another series of lines, the line rapidly overlap to create dark, dense lines of collagen.

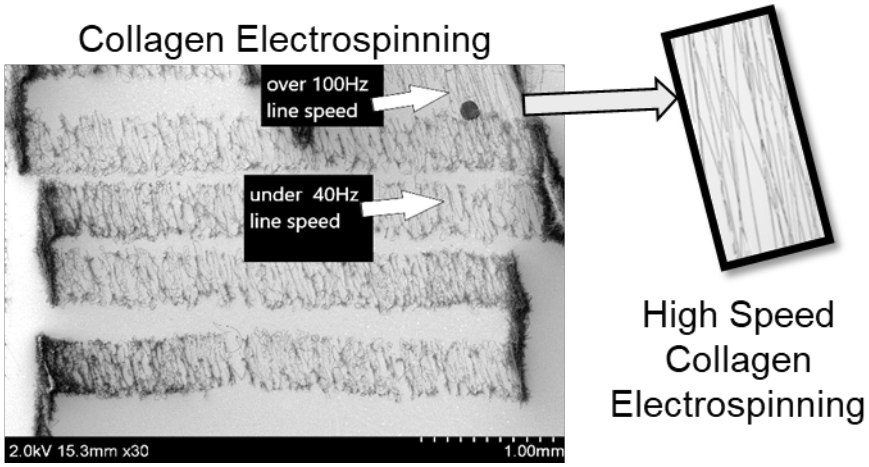


Figure 3. Scanning electron microscope image of electrospun collagen

1.2.1 Acquire and commission 3D bioprinter – Q3

We have procured the Cellink Model X bioprinter. We had two separate 1-day training sessions with the company representatives. We used the printer to print simple hydrogel structures to gain experience. We also printed hydrogel structures with fluorescent microspheres imbedded. We crosslinked the structures with calcium solutions. We have begun to print hydrogel structures with the Cellink bioprinter. We printed structures having 200 micron resolution, more than sufficient for our intended use.

1.2.2 Demonstrate 3D deposition of hydrogels onto electrospun collagen

Our research plan includes deposition of gel material onto the electrospun collagen fibers to fill in spaces between fibers and provide compression strength. As proposed GelMA is our primary candidate gel. GelMA is a gelatin with photopolymerizable methacrylate groups. GelMA is low cost, easy to process, and biocompatible. It has integrin binding motifs and matrix metalloproteinase sensitive groups which help living cells adhere, remodel, and migrate through GelMA. GelMA demonstrates an ideal temperature-sensitive shift in mechanical properties, which can be adapted to the 3D bioprinting process. We have used the BioX printer to deposit a pattern of GelMA (Cellink) onto electrospun collagen. Figure X shows that our current formulation of collagen swells in contact with GelMA. This is from insufficient crosslinking which is the work of the second project year in Task 1.2.3.


Figure 4

View of collagen fibers through a layer of GelMa printed onto the fibers. The striations in the image are collagen fibers that have absorbed liquid from the GelMa and swollen.

Specific Aim 2
1) major activities (accomplishments);

Specific Aim 2 is to successfully design, fabricate and 3D print microscale tracking units to provide intrinsic surgical motion and tissue response recording and feedback as an integral part of the surgical simulation system. In the first year of the program we focused on developing hardware prototypes of our active microsensors and on methods to deposit either our active or passive sensors into the gel-like tissue constructs we are developing under Specific Aim 1. We achieved a major milestone this year by producing a functional hardware implementation of our software model of the active microsensor communication protocol. We also demonstrated our novel method of depositing sensors using laser induced forward transfer to "shoot" microbeads into a gel.

2) specific objectives;

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
2.2.1 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

3) significant results

2.1.1 Design, assemble and evaluate FPGA based circuits for wireless nodal communication

Fabrication of a Microscale System-on-a-Chip A central theme to our approach is to use widely known commercially available technology whenever possible to reduce risk. Our use of focal plane gated arrays (FPGAs) is an example. FPGAs are microfabricated chips which can be programmed to form complex logic gate arrays. The program primarily connects available AND, OR, NOR, NOT and other logic gates to memory locations and counters, timers and switches. These connections become a fixed hardware implementation of the original algorithm written in MATLAB, Figure 11. The main benefit for our project will be the ease in which either the FPGA connection file or logic gate array layout can be microfabricated into a semi-custom chip called an ASIC.

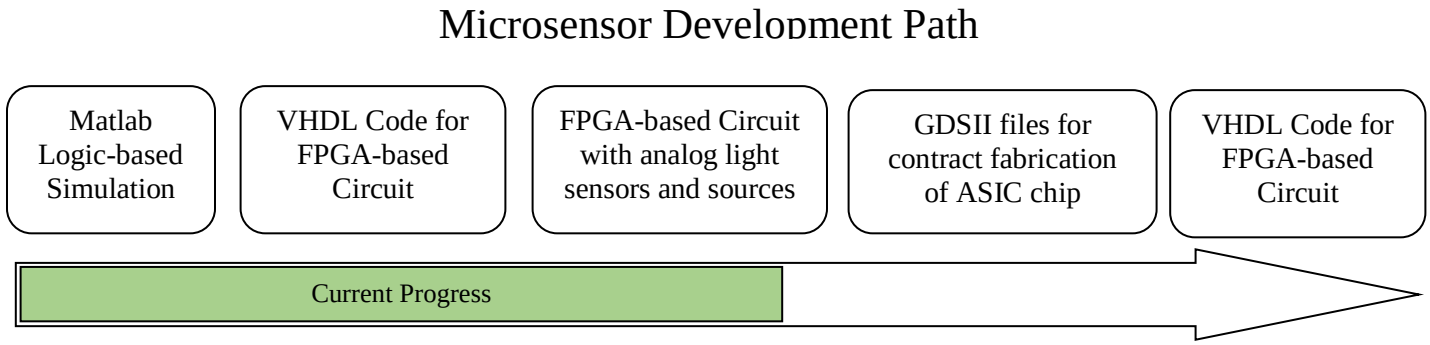


Figure 5.

Figure 5 shows the microsensor development path. We started with a Matlab computer simulation of an ideal series of sensors which can send data values in a self-organized "bucket brigade" style transmission network. The simulation was developed by Meadowave personnel prior to the start of the project. The first step was to transfer that simulation of a hardware code into true VHDL hardware code. After developing that code we transferred it to an FPGA chip-based circuit with optical components: LEDs, voltage amplifiers and light detectors, needed to transmit data in a real-world implementation. Figure 6 shows the output of VHDL code running on a PC. Four copies of the code were simultaneously running and were connected to each other in the following order: Chip 3 to Chip 2 to Chip 1 to Chip 0. The code was successfully run to transfer a value of 0 or 1 to the output (Chip 0). A prototype circuit was designed and then multiple copies assembled to demonstrate transfer of data from one chip/board to the next. Figure 7 shows four prototype boards transmitting data from one chip to the next. Any board can be removed and/or swapped, and the network will self-organize itself in less than 1 second. This is key in that in a surgical simulation tissue a scalpel might remove a microsensor from the network. Figure 7 shows via an oscilloscope readout from three boards, a successful transfer of a sensor value of 0 from Chip 1 to Chip 0 and then a value of 1 from Chip 2 to Chip 1 to chip 0.

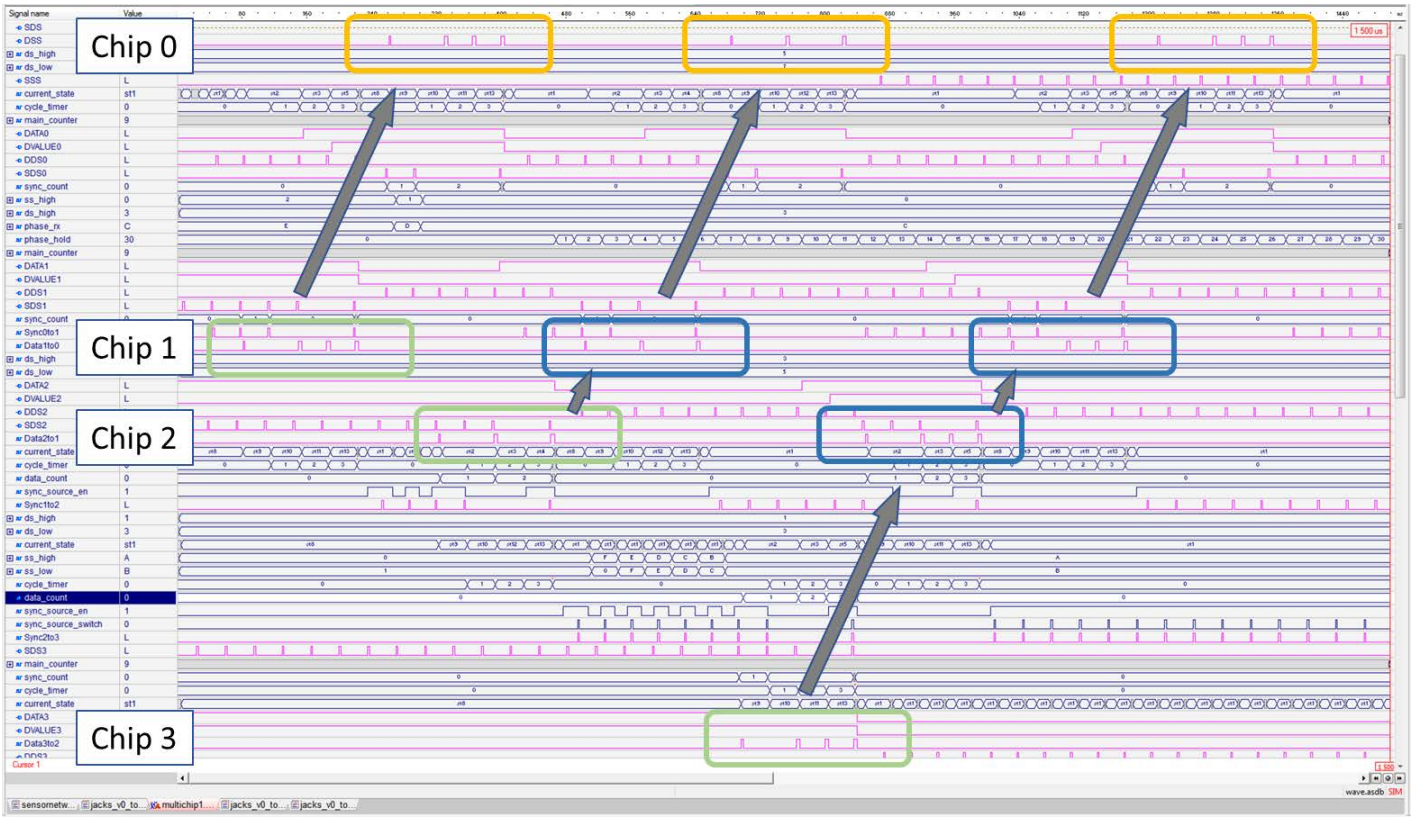


Figure 6. Development of VHDL code for a wireless sensor network communication algorithm. Fourchips are shown communicating. Each chip sends data optically to nearest neighbor until data is read from the last device (Chip 0). In each colored square, a pattern indicates a 0 or a 1 . Chips 1,2 and 3 start with data. Chip 1 sends a value of 1 to Chip 0, then Chip 2 sends a value of 0 to Chip 0 via Chip 1, finally Chip 3 sends a value of 1 to Chip 0 via Chips 2 and 1.

Figure 7. Prototype "breadboard" versions of sensor units. Four boards are shown communicating optically. The two large grids of LEDs provide a global clock signal for synchronization. Each board sends data optically to nearest neighbor until data is read from the last device. The order of boards can be changed, or a board removed and the network repairs itself to continue data readout.

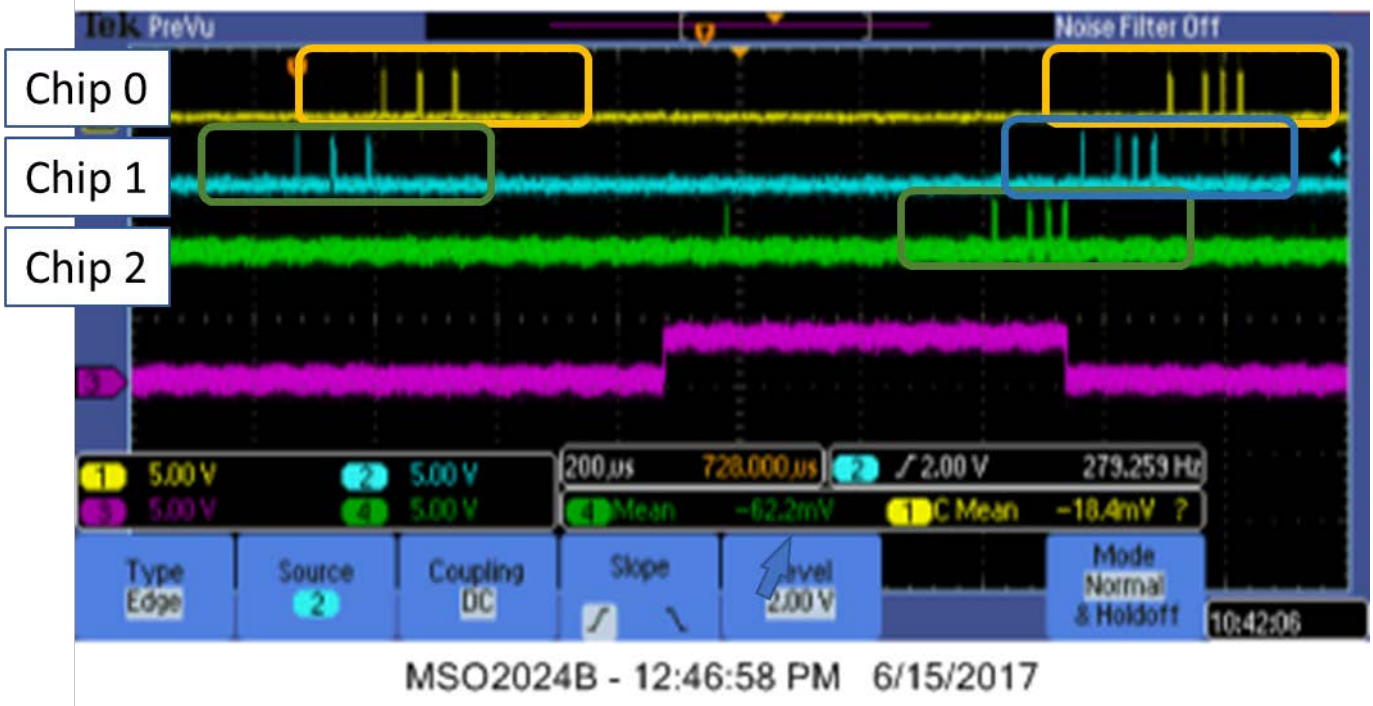


Figure 8 Oscilloscope voltage recordings show the transfer of a value of 0 from Chip 1 to Chip 0 then a transfer of a value 1 from Chip 2 to chip 1 to Chip 0.

2.1.2 Transfer FPGA design to ASIC microchips with bonded LEDs and photodiodes

In this subtask, the FPGA based circuit design from 2.1.1 will be translated into a custom chip. Our consultant, Dr Eisenstadt from the University of Florida, worked with us to find an electronics design automation software platform. We have selected Mentor Graphics, Inc as the software provider. We will use a version of their more affordable line called Tanner. The benefits of the Tanner software package are the ability to create both analog and digital circuits in one design and the ease with which one can use the VHDL code we have already created in subtask 2.1.1. We have received a quote that is within the budget parameters and we are currently testing the software in free "demo" format to be certain it will work for our needs.

Figure 9. A printed circuit board version of prototype sensor units measures 12mm x 16mm. This mini solar powered version of the boards will aid in converting the VHDL FPGA code to an ASIC under Task 2.1.2.

2.2.1 Deposition of 20, 40 and 100 micron microspheres and microchips into gel structures

A 266 nm laser was focused onto the titanium oxide layer of a BioLP™ "ribbon." The subsequent vaporization of this sacrificial layer causes beads immobilized on the surface to be ejected into the agarose gel positioned under the ribbon, penetrating the surface. Bead depth was determined using the microscope focusing adjustment scale.

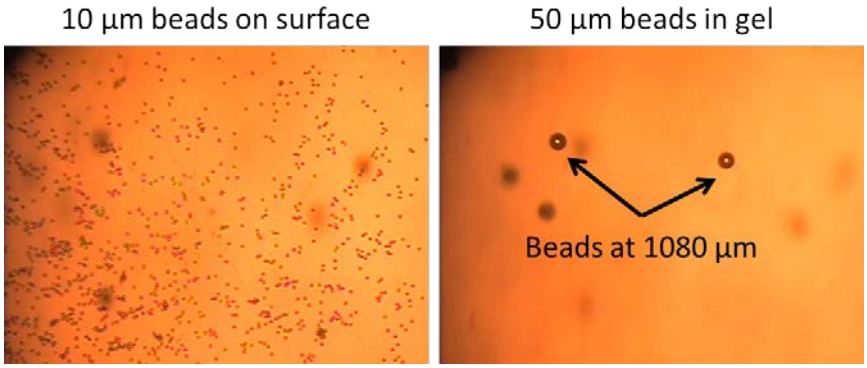
Agarose Gel and BioLP™ Ribbon Preparation

0.5% agarose gels were prepared by mixing 0.8 grams of agarose powder with 160 mL of water and microwaving the solution until the powder completely dissolved (approximately 45 seconds). The agarose solution was then poured into petri dishes and allowed to cool at room temperature (25°C) until gelation was complete and gels firmed. BioLP™ ribbons were prepared for experiments with beads of various sizes. Beads were first dispensed onto the ribbon in solutions of water to form a single monolayer and allowed to dry. Approximately 1 µL of a 1:1 solution of water and glycerol was applied to the beads to rewet them prior to forward transfer.

Bead Implantation via Bio-Laser Printing

A custom-built Bio-laser printer was utilized to implant beads from the ribbon into agarose gels. The construction of the BioLP™ has been extensively described in literature and will not be detailed here [4, 5]. Briefly, a Wedge-XF-266 nm solid state laser (Bright Solutions) was used at a 10 ns pulse width to irradiate the ribbon and generate material transfer from the ribbon. The ribbon consists of a 5 cm x 5 cm x 0.3 cm transparent quartz plate (Ted Pella, Inc., Redding, CA, USA) coated with an 85 nm layer of titanium dioxide (TiO_2). This TiO_2 layer serves as a sacrificial layer that absorbs the laser energy and transfers it into kinetic energy that propels the microbeads from the ribbon.

Agarose gels were then loaded onto the carrier substrate as described above and several bead sizes were shot into various agarose petri dishes at different laser power level.



2.2.2 Quantification of depth of penetration into gel structures and accuracy of placement

Measuring Peak Penetration of Implanted Beads

BioLP™ was utilized to implant beads of various sizes into 0.5% agarose gels. Briefly, a 266 nm laser was focused onto the titanium oxide layer of a BioLP™ "ribbon." The subsequent vaporization of this sacrificial layer causes beads immobilized on the surface to be ejected into the agarose gel positioned under the ribbon, penetrating the surface. A three-step protocol was used to quantify the depth of penetration into the gel. First, to visualize the surface of the gels, 10 µm beads were pipetted onto the gel to serve as a reference layer. Next, a microscope was focused onto the deepest layer of beads and the focal position on the microscope was recorded. Finally, the focus was then adjusted to the surface beads and difference in focal positions was recorded in order to calculate an estimate of penetration depth. Figure 1 illustrates the implantation of 50 µm beads at 100% laser power. It can be seen that beads penetrate into multiple depths within the gel as evidenced by the out of focus beads. The focal plane of these beads is between the surface and deepest beads. Beads ejected with insufficient force are deposited on the surface (not shown).

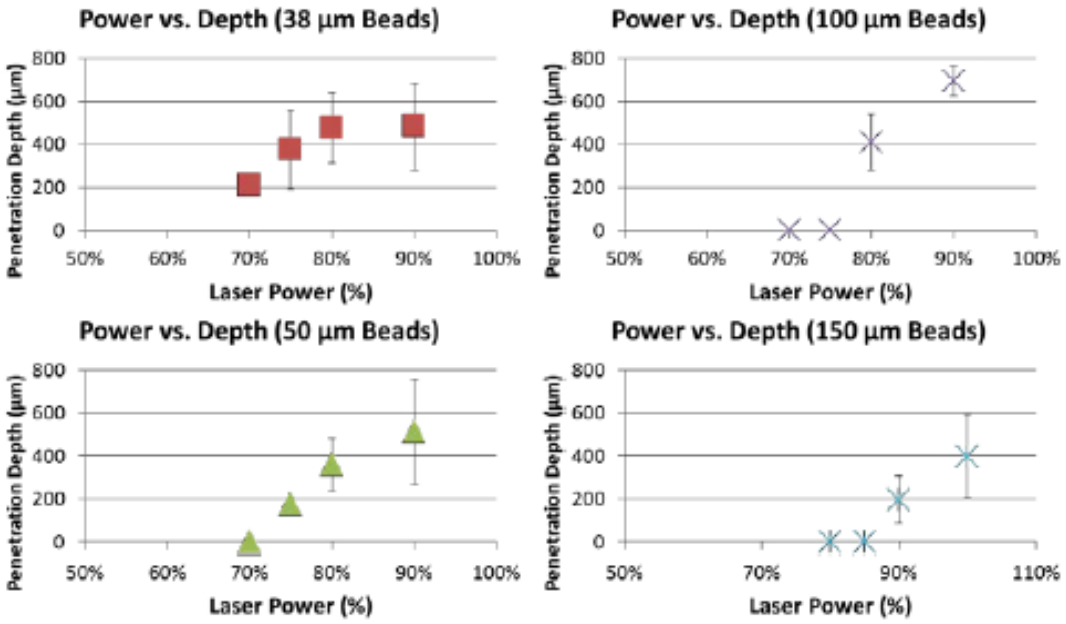


Figure 2 Laser power vs. penetration depth for 38, 50, 100, and 150 µm beads.

Bead size is plotted vs. average peek penetration depth for 90% laser power in figure 3. Despite 50 micron beads not penetrating gels at 70% (Fig. 2), they have similar penetration levels compared to 38 micron beads at 90%. The significantly larger mass of 150 micron beads is hypothesized to be the cause for lower penetration into gels.

Laser Power vs. Peak Penetration

Results of penetration measurements were gathered for various bead sizes and laser powers as documented in Table 1. First, the relationship between laser power and peak penetration was investigated for various bead sizes. In figure 2, several plots show the relationship between laser power and penetration depth for 38, 50, 100, and 150 micron beads. To record average peak penetration, the penetration depth of at least 3 separate implantation "shots" was recorded and the average and standard deviation calculated. Overall, 100 micron beads had the highest average peak penetration depth at 695 ± 69 µm at 90% laser power (336 µJ). The largest beads (150 µm) had an average peak penetration of 199 ± 109 µm, the smallest at 90% laser power. This may be due to the increased mass of beads which would require more energy to go further. It is also important to note that the average peak penetration of 38 micron beads seem to saturate at 80% laser power.

Discussion of stated goals not met

All proposed project year 1 goals have been met. However, while we have printed a few layers of collagen under task 1.1.2 , we will pursue printing20+ layers of collagen fibers . To date, we have printed multiple layers of PEO and expect easily translation into printing multiple layers of collagen in April 2018. We also have begun to assemble the mini boards shown in Figure x under task 2.1.2. Thistask is on schedule-- to be completed by the second quarter of year 2. We expect to finish assembling the mini solar powered boards by April 2018.

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

"Nothing to report ", however, we are scheduled a medical student from USUHS to assist with the project in April 2018.

How were the results disseminated to communities of interest?

Abstracts submitted for MHSRS 2018.

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

During the next reporting period,, we will fabricate 400-1000 micron collagen-based tissue constructs to can test the mechanical properties. Additionally, we will transfer the sensor design from circuit boards to a first version custom fabricated chip. This willby accomplished byfocusing on the following tasks from our statement of work listed below.

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
1.2.3 Demonstrate crosslinking of electrospun collagen and 3D bioprinted hydrogels and determine Young's Modulus
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

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
2.3.3. Quantification of deposition into collagen/gel ophthalmic constructs
2.5.1. Design and acquisition of camera based optical particle tracking system and software
2.5.2. Demonstration of particle location and tracking on dry surface
2.5.3. Demonstrate particle location and tracking in gel matrix

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

"Nothing to Report."

What was the impact on other disciplines?

"Nothing to Report."

What was the impact on 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 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:

• Publications, conference papers, and presentations

MHSRS abstracts submitted for 2018.

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

Research is part of DOD website for bioprinting efforts. https://www.usuhs.edu/4dbio3

• 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

"Nothing to Report."

• Other Products

As this is the first year of the project none of our products have arisen to the level of making a meaningful contribution towards the understanding treatment of ophthalmic patients via training of ophthalmology residents. However, we have produced a VHDL algorithm for hardware programming of the field programable gated array logic chip which should lead to training benefits by year 3 of the project and 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: 0.5
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: 5.2
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: 2

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

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: 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

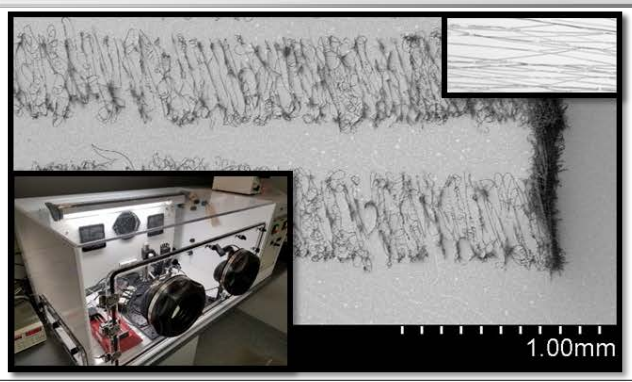
Log Number: BA150090
Contract Number: W81XWH-17-C-0003

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.



Our direct write electrospinning 3D printer (lower image) can place layers of oriented 0.4 mm long collagen fibers similar to corneal lamellae (background). Higher speeds result in straight 3 micron thick fibers(upper image).

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**	

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: $210,500
Actual Expenditure: $382,748.33