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14. ABSTRACT

The report herein describes progress made in the first year of EMERGE: Engineered Materials the Create Environments for ReGeneration via Electric Fields. The objective of the project is to develop a next-generation ocular implant material with biomimetic chemistry and nanotopography with unique drug delivery functionality to 1) provide appropriate biochemical and biophysical cues for enhanced cell migration, differentiation and proliferation and 2) deliver wound electric field enhancing pharmaceuticals for enhanced bioelectronics cues. To this end, we are developing a collagen material with tailored nanotopography with ability to deliver endogenous wound electric field-enhancing molecules that will encourage and accelerate the wound healing process. We monitor wound electrophysiology as a function of time using a vibrating probe.

15. SUBJECT TERMS
Corneal, Wound healing, Collagen, Aminophylline, Nanoparticles, Drug delivery

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1. Introduction

The *objective* of the project discussed here is to develop a next-generation ocular implant material with biomimetic chemistry and nanotopography with unique drug delivery functionality to 1) provide appropriate biochemical and biophysical cues for enhanced cell migration, differentiation and proliferation and 2) deliver wound electric field enhancing pharmaceuticals for enhanced bioelectric cues. To this end, we are developing a collagen material with tailored nanotopography with ability to deliver endogenous wound electric field-enhancing molecules that will encourage and accelerate the wound healing process. The material will be engineered to closely mimic the *in vivo* cornea environment, exhibiting excellent transparency and mechanical strength, properties important for high performance cornea implants. Incorporation of drug delivery functionality both directly into the collagen membrane, and in embedded nanoparticles, will allow tailored, sustained delivery of bioelectric modulators that have been shown by our team to stimulate cell responses related to the healing process. To reach our goal, our research is organized around four aims. Aim 1 is to correlate the presence and dosage of the bioelectric modulators with wound healing to determine formulae for maximizing wound EF. Aim 2 is to develop the collagen material and correlate the relationship between collagen fiber topography and wound healing to enable optimization of the material structure. Aim 3 is to incorporate the bioelectric modulators into the collagen membrane and characterize the release. Aim 4 is to evaluate and optimize the therapeutic benefit of collagen materials containing drug-loaded nanoparticles on corneal healing. The technology developed in this project will be applicable for the treatment of multiple types of injuries to ocular structures and could be extended to introduce additional pharmaceuticals, such as antibiotics and analgesics to target different aspects of ocular injury. An implantable EMERGE biocomposite would facilitate accelerated wound healing, ensure a faster return to full function, and improve the quality of life for those inflicted with eye injuries.

2. Keywords

(Limit of 20 words)

Wound healing
Cornea
Bioelectric
Collagen
Drug delivery
Vibrating probe
Endogenous electric field
Nanoparticles
Poly (lactic co-glycolic acid) (PLGA)
Injury
Aminophylline

3. Accomplishments

Major goals and objectives

The overall objective of EMERGE is to develop an ocular implant material with biomimetic chemistry and nanotopography and unique therapeutic delivery functionality to improve corneal wound healing through two strategies:

1. Providing biochemical and biophysical cues for enhanced cell migration, differentiation and proliferation.
2. Delivering chemical bioelectric modulators for enhanced wound electric field.

To realize this objective, the project is based around four Aims, each with an associated Task, as described in the Statement of Work (SOW). These Tasks are as follows:

1. Correlate the presence and dosage of the bioelectric modulators with wound healing.
2. Develop a collagen-collagen biocomposite material with tailorable nanostructure and collagen fibril alignment, and correlation of the relationship between collagen fiber topography and wound healing to enable optimization of the biocomposite structure.
3. Incorporate bioelectric modulators into a pharmacological delivery system within the collagen-collagen composite and characterize release.
4. Measure endogenous electric field (EF) at corneal wound sites and correlate with effects of bioelectric modulators, pharmacological delivery system, and collagen biocomposite to assess efficacy of these materials and pharmaceuticals for healing.

Each Task has smaller subtasks (specific aims); also called out in the SOW. Goals in year 2 have focused on these subtasks, ensuring progress towards achieving our overall project objective.

Accomplishments

Accomplishments are discussed below, organized by Aim. Specific Aims relevant to work during the second year of the project are identified.

Aim 1

Specific Aim 1a: Measure wound electric field (EF) and ion fluxes at corneal wounds with a vibrating probe and ion selective electrodes.

To summarize what has been reported previously, we have characterized the spatiotemporal dynamics of rat cornea wound electric currents and ion flux using the vibrating probe and ion-selective electrodes, respectively. For both shallow (~30μm) and deep (100μm) wounds, electric current and ion flux are maximized at

the wound edge, smaller at the wound center, and reduced to normal (unwounded) values a small distance outside the wound. Moreover, time-lapse measurements of electric current in the hours after wounding indicate that the wound electric signal is long-lasting and appears to be actively-regulated. The wound electric signal increases rapidly until approximately 40 minutes after wounding and is maintained at a high level for several hours. To generate reproducible deep wounds (100μm), a laser was used and settings were optimized. Deep laser wounds take longer to heal than shallow epithelial wounds, but did heal 100% by day 8 in comparison with shallow epithelial wounds that heal quite quickly (48 hours).

Specific Aim 1b: Quantify effects of pharmacological agents on corneal wounds and resulting EF using rat corneal model.

We have chosen to focus on the use of aminophylline as a bioelectric modulator to enhance wound healing. To ensure that the aminophylline has the same effect on deep wounds as shallow epithelial wounds, we measured wound electric field using the vibrating probe with and without the presence of 1 and 10mM aminophylline. The presence of aminophylline appears to result in an increase in the wound electric field and correlates with the aminophylline concentration (Figure 1).

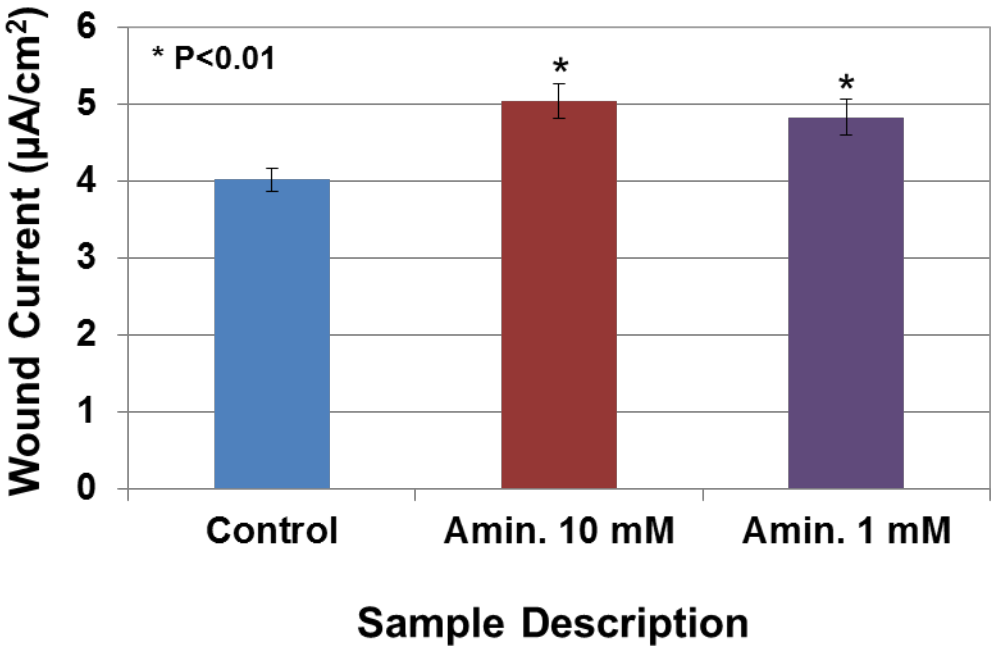


Figure 1: In a rat cornea model, aminophylline (1 and 10 mM) increased cornea wound electric signal.

Specific Aim 1c: Using the human cornea test platform, determine optimal concentrations & combinations of pharmacological agents for regulating wound electric signals to maximally stimulate the endogenous EF and thus facilitate wound healing and regeneration of injured cornea.

As indicated previously (Annual Report Year 1), the rat model shows a dynamic time course of rising and maintained electric current similar to that of a human cadaver eye, so the rat model has been the focus of work on this Aim to date. Figure 2A shows wound healing of a deep wound (100µm) without and with the presence of 1mM aminophylline as function of time. Optical coherence tomography (OCT) images of a control deep wound as well as a deep wound treated with the bioelectric modulator aminophylline are shown in Figure 2B and 2C. Figures 2 and 3 show that the presence of 1mM aminophylline facilitates wound healing and regeneration of an injured cornea in the deep wound faster than the control case.

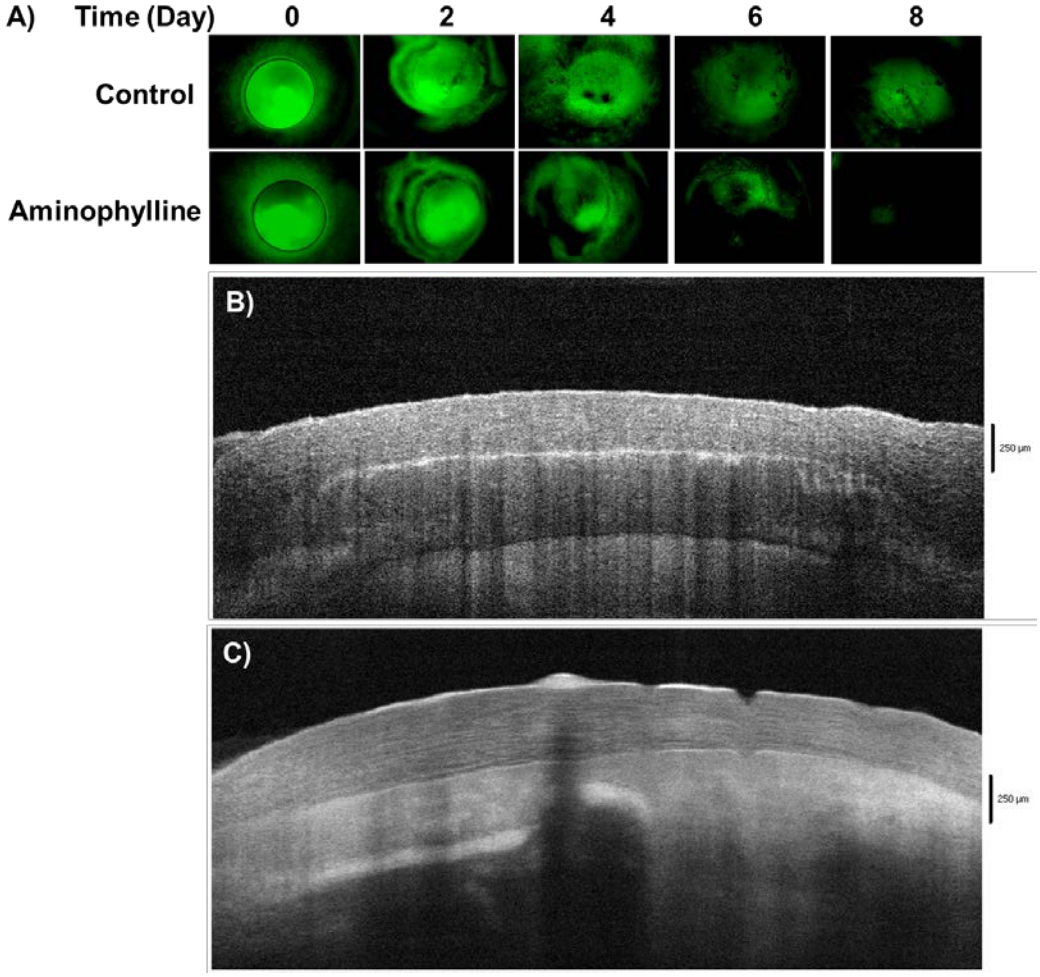


Figure 2: (A)Wound healing of a deep wound (~100µm) without and with the presence of 1mM aminophylline as function of time. Wounds were visualized with fluorescein dye. The wound is the bright area in the center. Optical coherence tomography (OCT) images of a deep wound (B) control and (C) aminophylline (1mM) treated cornea at day 8 showing significant more healing for the cornea treated with the bioelectric modulator aminophylline.

Figure 3 shows the percentage of wound healing as function of time for corneas treated with the bioelectric modulator aminophylline (1mM). The untreated corneas showed only 40% wound healing in comparison with the treated corneas that

showed significantly more healing (~ 70%). These results indicate the importance of the bioelectric modulator aminophylline in the healing of deep wounds.

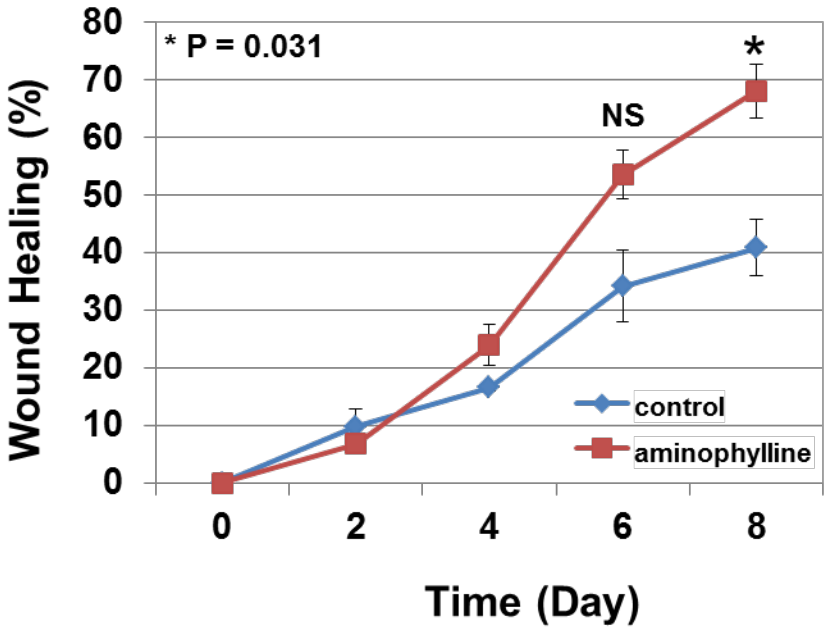


Figure 3: In a rat model, percentage of wound healing as function of time for deep wound showing significant more healing for the cornea treated with 1mM aminophylline (Control, N = 4 and Aminophylline, N = 5). NS = No Significant Difference (P > 0.05)

We have made significant progress toward understanding the effect of the bioelectric modulator aminophylline on the healing of deep wounds as well as the effect of aminophylline dosages and the maximization of endogenous EF.

Aim 2

Specific Aim 2a: Synthesize collagen-collagen composites consisting of electric-field aligned collagen vitrigels and electrospun collagen nanofibers.

We developed a process for the fabrication of engineered collagen membranes (Figure 4). The fabrication parameters can be controlled to tailor fiber diameter, fiber density, thickness-normalized transparency and suture strength. This novel fabrication method allows tailoring of collagen membrane thickness and area. This fabrication process was used in combination with a Design of Experiment (DOE) to synthesize collagen membranes with tailorable properties.

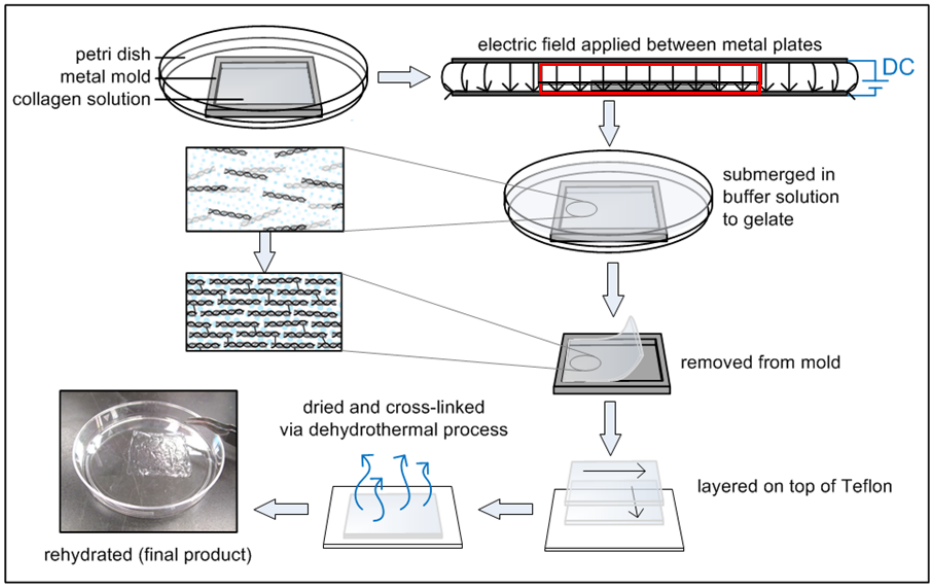


Figure 4: Schematic of the fabrication process of the collagen membranes under the influence of an electric field. The red box represents the petri dish.

Specific Aim 2b: Characterize strength and transparency of collagen-collagen composites and correlate with synthesis parameters and material structure.

The fabrication and characterization of the DOE have been completed. Table 1 summarizes DOE factors per run and measured optical and mechanical properties for the collagen membranes.

Table 1: Summary of DOE factors per run and measured optical and mechanical properties for the collagen membranes.

Run	Collagen Concentration (mg/mL)	Dialysis	Electric Field (V/cm)	Electric Field Time (Hours)	Processing Temperature (˚C)	pH	Normalized Transmittance at 550 nm (%) (150 µm)	Suture Strength Normalized Max Load x 10^{-4} (N/µm)
1	10	No	10	8	37	2.5	83 ± 4	2.4 ± 0.2
2	10	Yes	5	8	37	4	92 ± 4	3 ± 1
3	5	Yes	5	8	4	2.5	97 ± 5	2 ± 1
4	5	Yes	5	4	37	1	73 ± 6	2 ± 1
5	10	No	5	4	4	4	92 ± 8	1.1 ± 0.3
6	10	Yes	10	4	4	2.5	96 ± 9	0.9 ± 0.2
7	10	No	5	8	23	1	76 ± 6	2 ± 1
8	10	Yes	10	4	23	1	72 ± 5	2 ± 1
9	5	No	5	4	23	2.5	87 ± 4	2 ± 1
10	5	No	10	4	37	4	78 ± 12	3.1 ± 0.5
11	5	Yes	10	8	23	4	90 ± 3	4 ± 1
12	5	No	10	8	4	1	64 ± 4	3 ± 1

The suture strength of each collagen membrane was measured with an Instron 5942 with a 500N load cell, as previously reported (Annual Report Year 1). For some synthesis parameters the suture strength of the collagen membranes significantly exceeded the suture strength of collagen materials reported in the literature[1] (LIT,

Figure 5A) and our previously developed collagen vitrigels (CV, Figure 5A). In this study, the suture strength of the collagen membranes ranged from 0.9 to 7 x 10^{-4} N/µm. The statistical analysis revealed collagen concentration (Prob. <0.0001), pH (Prob. 0.0153), and the interaction of collagen concentration and pH (Prob. 0.0385) as influential factors for the suture strength (normalized load). Suture strength is dependent on collagen concentration, processing temperature and pH, as can be observed by the trend lines in Figure 5B. For the maximization of suture strength, lower collagen concentration, higher processing temperature, and a pH of 2.5 are preferable.

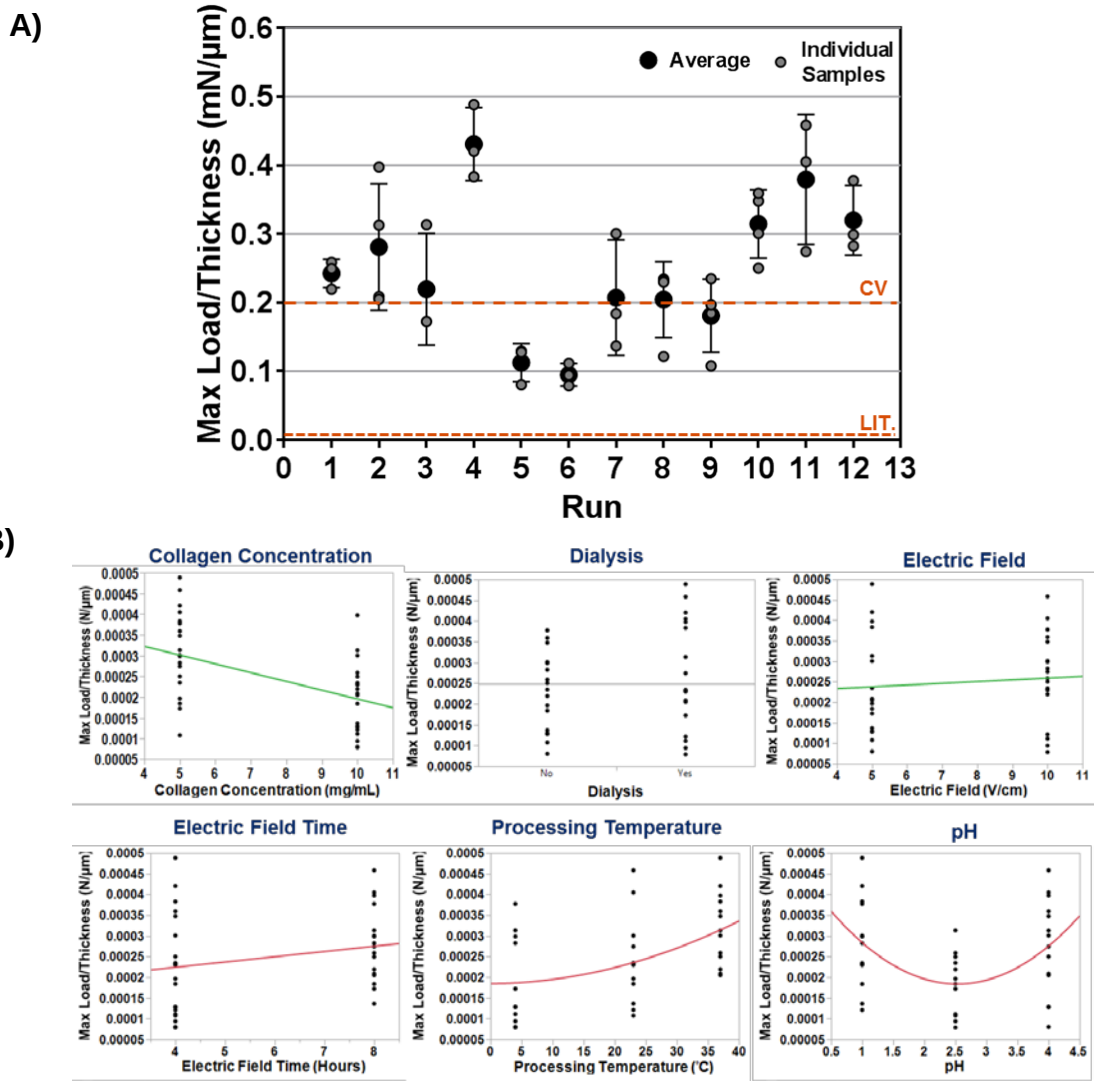


Figure 5: A) Maximum load per thickness as function of DOE run. B) Maximum load per thickness as function of DOE factors showing the trends for the suture strength (normalized load).

[1] 1. Li et al. "Recruitment of multiple cell lines by collagen-synthetic copolymer matrices in corneal regeneration," Biomaterials (2004).

Depending on the fabrication factors, the normalized transmittance of the collagen membrane ranged from 64 to 97% (membrane thickness 150µm), which exceeds the transmittance of collagen membranes reported in the literature[1] or CVs (Figure 6A). The statistical analysis revealed pH (Prob. < 0.0001), collagen concentration (Prob. 0.0015), electric field (Prob. 0.0160), electric field time (Prob. 0.176), and the interaction of collagen concentration and electric field (Prob. 0.0001), and collagen concentration and processing temperature (Prob. 0.0333) as influential factors for the normalized transmittance. For the maximization of transmittance, a pH of 2.5, higher collagen concentration, higher electric field strength, and longer electric field time are preferable.

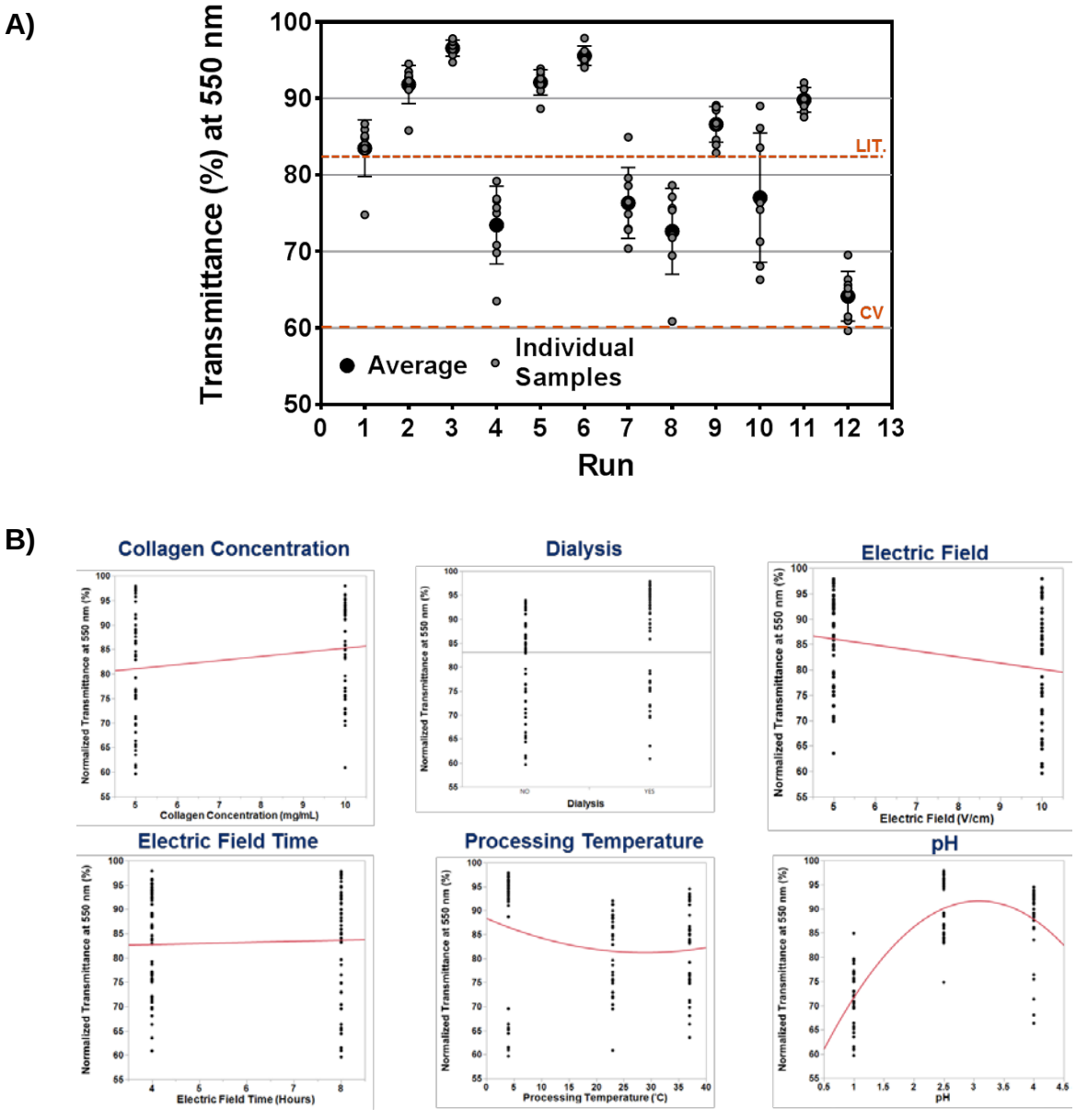
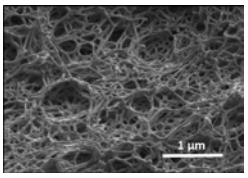
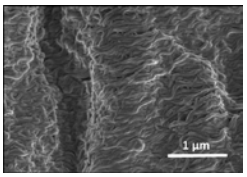
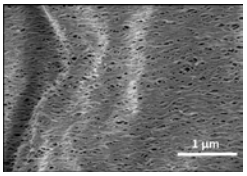


Figure 6: A) Normalized transmittance as function of DOE run. B) Normalized transmittance as function of DOE factors showing the trends for the transmittance.

Specific Aim 2c: Down-select to composite configuration (s) with a minimum thickness of 500 µm, transparency of 75% (to maintain vision) and suture strength (corresponding to a break point of at least 0.2 mN/µm), to ensure ease of application and integrity of wound closure).

In order to down select which collagen membranes to utilize for the remainder of the project, optimization of both optical and mechanical properties was of critical importance. Table 2 summarizes the down selected collagen membranes. The down selection was performed taking into consideration the fact that maximizing the properties might not result in the most effective material for the application. The thresholds used for the down selection to ensure the appropriate balance of the material properties are transparency of 75% (to maintain vision) and suture strength (corresponding to a break point of at least 0.2 mN/µm), to ensure ease of application and integrity of wound closure). All the selected materials met and exceeded the thresholds for the optical and mechanical properties. Moreover, the morphology and topography was taken in consideration for the down selection, where different morphologies and topographies will be studied as seen in Table 2.

Table 2: Summary of down-selected collagen membranes with different morphologies/topography and properties.

Sample	Sample Processing (Factors)	Normalized Transmittance at 550 nm (%)	Suture Strength x 10^{-4} (N/µm)	Morphology/ Topography
Control	10 mg/mL collagen concentration (pH 2.5), dialyzed solution, no electric field is applied	89 ± 4	3.7 ± 0.4	
EF 1	10 mg/mL collagen concentration (pH 2.5), dialyzed solution, applied 5 V/cm electric field for 8 hours under the influence of EF at 23˚C	94 ± 2	1.5 ± 0.9	
EF 2	5 mg/mL collagen concentration (pH 4), dialyzed solution, applied 10 V/cm electric field for 8 hours under the influence of EF at 23˚C	90 ± 3	4 ± 1	

Specific Aim 2d: Use the vibrating probe and ion selective electrodes to map the electrical signal at the cornea wounds (rat model) and variations resulting from application of the collagen composite (with varying topography) to the injured area. Particular attention will be focused on characterizing the collagen materials specific intrinsic properties (e.g. electrical conductivity in hydrated collagen).

Using the vibrating probe technique, we correlated the effect of collagen topography on endogenous EF (wound electrophysiology) and wound healing. Figure 7 shows a schematic of the wound, vibrating probe and collagen membrane placement. The characterization of wound electric signal was performed using a 2mm diameter, 100μm thick collagen membrane material; this was placed on the wound. The samples used in the experimentation include: control (wound, no collagen membrane in place), non-aligned collagen membrane (control, NA) and electric field-aligned collagen membrane (material made under the influence of an electric field, EF1). A separate wound electrophysiology characterization was performed after securing the collagen membrane in place using fibrin adhesive.

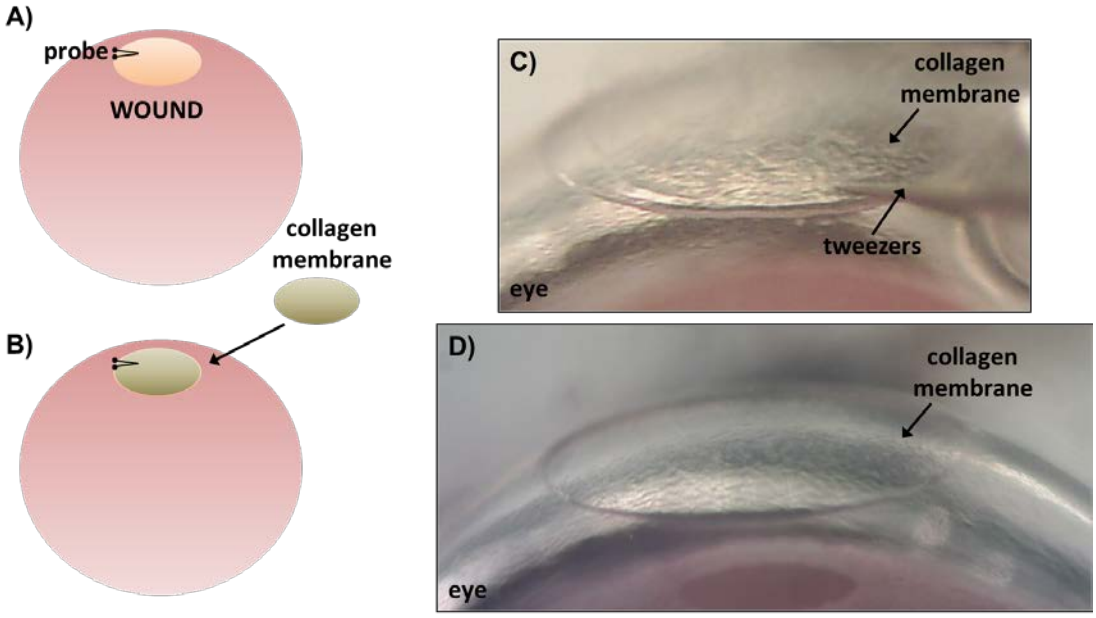


Figure 7: Schematic of the A) laser deep wound and vibrating probe. B) Placement of the collagen membrane on the deep wound. C and D) Images of collagen membrane placed on the wound bed.

The wound current, given the presence of any collagen membrane on the wound bed, was independent of collagen topography. However, the collagen membrane does not prevent the measurement of the wound current (Figure 8A). Likewise, securing the collagen membrane with the fibrin adhesive does not prevent the measurement of the wound current (Figure 8B). Therefore application of the membrane results in an insignificant reduction of wound current in comparison with the control (i.e. deep wound). Any current reduction is due to the distance of the vibrating probe during the measurement. The collagen membrane does not

reduce the ion transport. These results are promising because the monitoring of the wound healing progress using the vibrating probe will be possible.

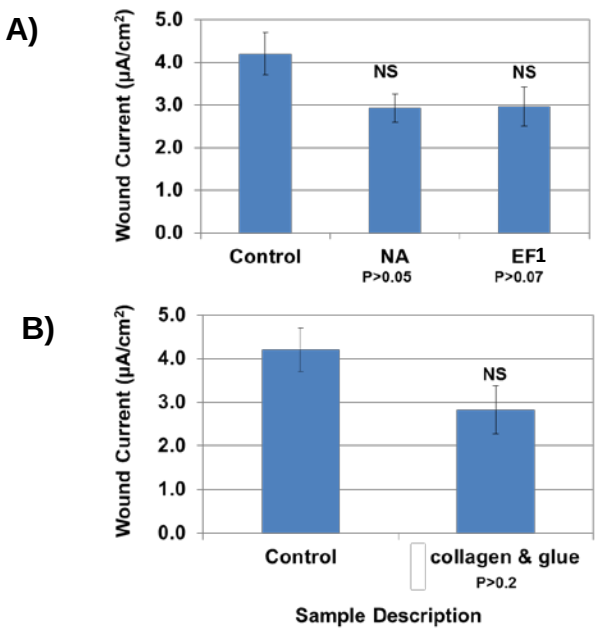


Figure 8: A) Wound current as function of the presence of different collagen membrane topologies. The presence of the collagen membrane on the wound bed caused a small, but insignificant reduction of measurable wound current. B) Wound current for collagen membrane secured using fibrin adhesive showing small effect on the wound current. In this case, the control is a deep wound with no collagen membrane present.

Specific Aim 2e: Correlate collagen composite topography with effects on endogenous cornea EF and down-select collagen biocomposite topography to maximize endogenous EF stimulation, allow targeted ionic transport to the migrating wound edge, and facilitate healing.

The ability to monitor the wound healing process is crucial in determining the effects of collagen membrane topography, bioelectric modulation (i.e. use of aminophylline), and any combination of these treatments. To study the effect of the collagen membrane during the wound healing assay, we placed a drop of the fibrin adhesive over the wound to hold the collagen membrane in place, as previously described. We utilized fluorescein dye to monitor the wound healing progress; when the fluorescein dye is in contact with the wound bed, a fluorescent signal is observed (Figure 9A). No fluorescent signal or label is observed when the collagen membrane material is in contact with the fluorescein dye (material alone, Figure 9B). Furthermore, the collagen membrane material secured with the fibrin adhesive on the wound bed does not prevent the observation of the fluorescent signal when the fluorescein dye is added (Figure 9C).

Figure 9: Fluorescent characterization of A) deep wound in contact with the fluorescein dye showing the fluorescent signal by labeling the wound bed. B) Collagen membrane in contact with the fluorescein dye showing only very slight fluorescent signal. C) Collagen membrane held in place on the wound bed by fibrin adhesive. The fluorescein dye diffused through the fibrin and collagen membrane to label the wound.

Collagen membranes with varying topography were applied to superficial wounds to understand effects of topography on healing. In this study, four eyes were studied: two controls with no membrane applied, and one each of non-aligned collagen membrane (NA) and electric field-aligned collagen membrane (EF1) applied. All four eyes healed within 48 hours (Figure 10A). Interestingly, the eye with the NA material healed slowly in the first 24 hours. However, the same eye was able to heal within 48 hours similar to the control and EF material. Using similar camera settings, wound labeling at 24 hours was not observed for the eye with the NA material (Figure 10B). The collagen implants appeared to still be in place after 48 hours. Future work will be focus on similar experimentation with the deep wounds and corresponding wound healing assay.

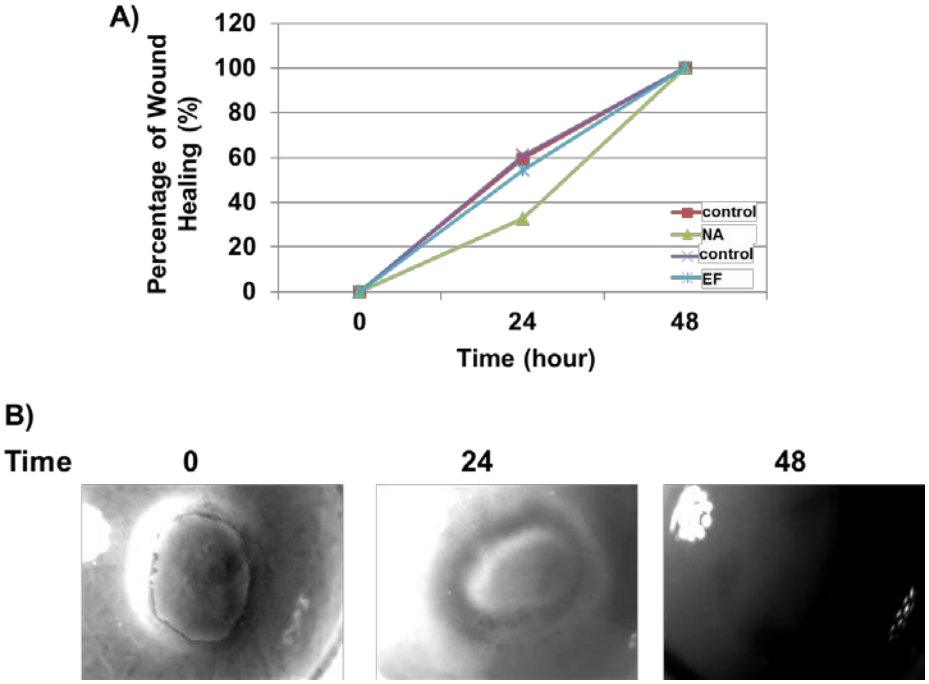


Figure 10: A) Percentage of wound healing as function of healing time for different collagen membrane topographies. B) Fluorescent microscopy of NA collagen membrane showing wound labeling as function of time.

Aim 3

Specific Aim 3a: Synthesize nanocarriers for encapsulation and delivery of bioelectric modulators, as required based on Tasks 1b & c; and Specific Aim 3b: Encapsulate bioelectronics modulators in nanoparticles and characterize modulator loading, release kinetics and drug stability.

We chose to focus on optimization of aminophylline release, a bioelectric modulator with well-demonstrated effects on endogenous electric field and wound healing. In order to achieve the delivery of aminophylline, we are pursuing a two-component biomaterial design for combined burst and extended therapeutic release. Using this approach, the collagen membrane will provide a burst release and poly(lactic-co-glycolic) acid (PLGA) therapeutic-loaded nanoparticles will provide the extended and controlled release (Figure 11).

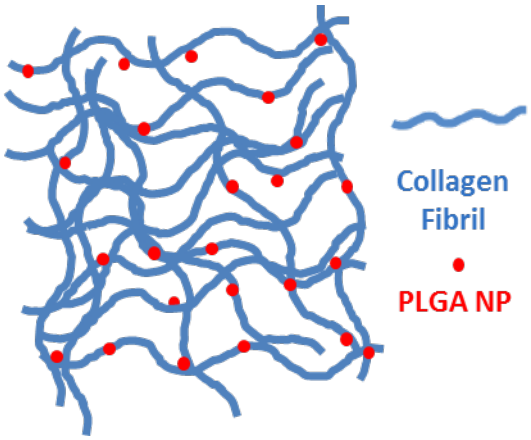


Figure 11: Design of two-component biomaterial for burst and extended therapeutic release.

We have performed preliminary characterization of the release of aminophylline directly from the collagen membrane material. The preliminary results showed a burst release of the therapeutic within 24 hours. On the other hand, encapsulation of aminophylline in PLGA, a biocompatible material approved for use in implants by the Food and Drug Administration (FDA), results in a sustained deliver. When aminophylline is encapsulated in a PLGA shell, gradual erosion of the shell through a hydrolysis mechanism and therefore gradual release of aminophylline is achieved. Therapeutic release profiles can be tailored by varying both the PLGA polymer used to produce the nanoparticles, and the nanoparticle loading concentration into the collagen membrane.

The aminophylline loaded PLGA nanoparticles were made through a process known as double emulsion solvent (DES) evaporation. For this process, an aqueous buffered solution of aminophylline of known concentration was prepared. Separately, a solution of PLGA and Pluronic F68 in ethyl acetate was prepared. The aminophylline solution was transferred into the PLGA solution and the mixture was briefly sonicated. During sonication, the solution is kept over ice to avoid

overheating. The resulting water in oil emulsion was placed into a second aqueous buffered bath with Pluronic F68. The mixture was again briefly sonicated. The resulting water/oil/water emulsion is placed into a rotary evaporator to remove ethyl acetate, so as to form solid nanoparticles. Following solvent removal, the solution is purified through a tangential flow filtration method. The purified aqueous nanoparticle solution was then lyophilized, so as to afford dry powder with increased shelf-life (Figure 12). Characterization of the aminophylline release from nanoparticles will be performed by a third party laboratory for non-biased therapeutic release characterization using liquid chromatography-mass spectrometry (LC-MS).

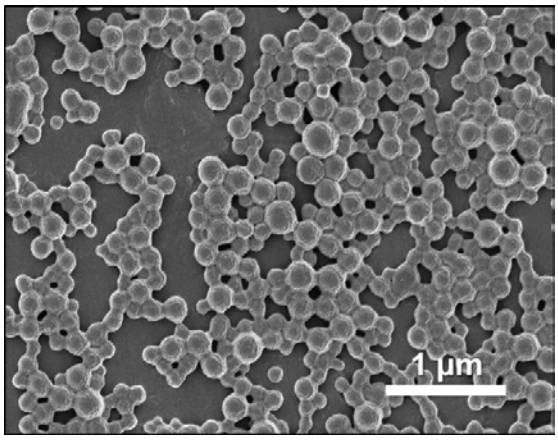


Figure 12: Scanning electron micrograph (SEM) of PLGA nanoparticles.

So as to determine the tuneability of the therapeutic release from the PLGA nanoparticles, two different molecular weights (7-17 kg/mol and 38-54 kg/mol) were utilized for distinct nanoparticle syntheses. The molecular weight of the PLGA polymer directly affects the kinetics of the erosion mechanism for the nanoparticle shell (Figure 13). The higher molecular weight PLGA polymers yield slower shell erosion, whereas lower molecular weight polymer yield a faster erosion process. The speed of the PLGA erosion is directly related to how quickly the therapeutic is released from the nanoparticles. The ability to easily alter the therapeutic release profile by changing the polymer molecular weight represents a means to tailor the material for the treatment of ocular injuries.

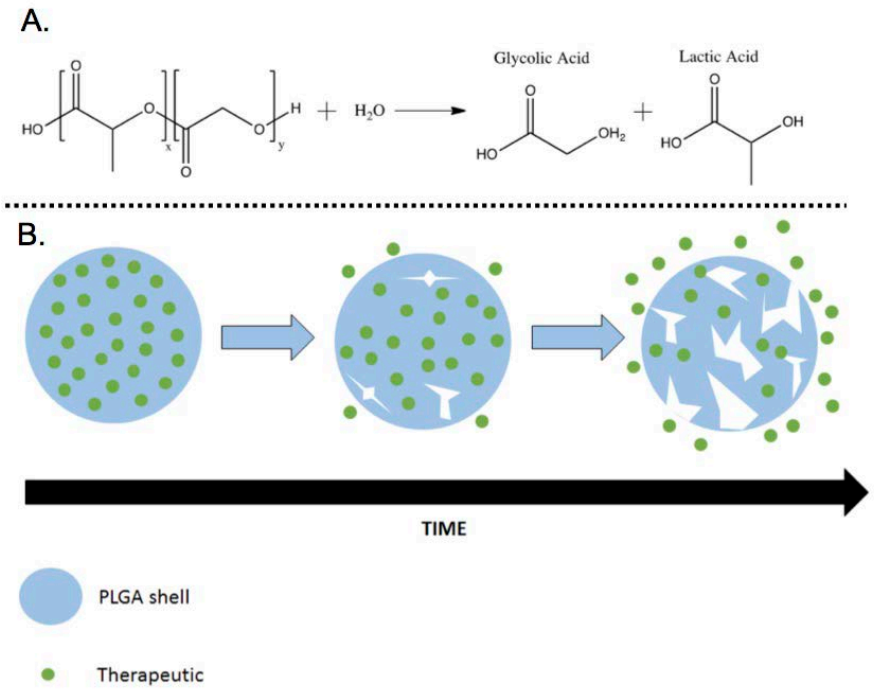


Figure 13: A) Mechanism of hydrolysis of PLGA nanoparticles, and B) schematic showing the release of entrapped therapeutic from a PLGA particle.

Once the therapeutic release profiles from both the nanocarriers and the collagen membrane are fully understood, then composite materials composed of collagen membranes loaded with PLGA nanoparticles will be made. Knowledge of the therapeutic release rates from both the collagen membrane and PLGA nanoparticles are important, because it allows one to effectively engineer implantable materials that can have both a burst and sustained therapeutic release. This dual nature therapeutic release is necessary because experiments from UC-Davis have shown that the need for both a burst and sustained therapeutic release to effectively heal corneal injuries.

Opportunities for training and professional development

Nothing to report for Year 2.

We plan for some project contributors to attend conferences, listed in the Dissemination of Results section.

Dissemination of Results

Nothing to report for Year 2.

We plan to publish a paper describing the aminophylline-loaded PLGA nanoparticle synthesis in year 3. We will also assemble a manuscript on electric field enhanced collagen membranes, and wound healing of stromal wounds in year 3.

We have a goal of disseminating results at a technical conference in year 2. We will consider submitting abstracts to at least one of the following:

- The Association for Research in Vision and Ophthalmology, ARVO 2017, May 7-11, 2016
- The Materials Research Society Spring Meeting MRS Spring 2017, April 17-21, 2017
- Biomaterials & Tissue Engineering (GRS), Gordon Research Conference, July 22-23, 2017

Future plans to Accomplish Goals and Objectives

For the next reporting period, we will focus on the following:

- Examining wound healing of deep wounds and wound healing assay with collagen membranes present.
- Examining wound healing with collagen membranes present, releasing aminophylline
- Characterization of aminophylline release from collagen membrane and from synthesized nanocarriers, separately and as a system.

4. Impact

EMERGE is aimed at developing a next-generation ocular implant material with biomimetic chemistry and nanotopography with unique therapeutic delivery functionality to 1) provide appropriate biochemical and biophysical cues for enhanced cell migration, differentiation and proliferation and 2) deliver wound electric field enhancing pharmaceuticals for enhanced bioelectronics cues. We are using a unique characterization tool, the vibrating probe, to measure the endogenous electric field at the wound edge. The novel aspects of this project keep us at the forefront of biomaterials (specifically collagen-based biomaterials) and wound healing as it relates to endogenous electric fields (and their manipulation). To the best of our knowledge, we are the first to synthesize aminophylline loaded PLGA nanocarriers, and to combine these nanocarriers with a uniquely engineered collagen material. We have filed internal IP Disclosures at JHU/APL to protect IP related to the electric field engineered collagen membranes as well as the aminophylline-loaded nanocarriers. (IP disclosures have been filed with iEdison.)

A successful project will have a considerable benefit to both the military and society at large. Despite representing only 12% of the body's surface area, the head, face and neck body region encounters 30% of combat wounds, an increase from prior conflicts.[2,3] Ocular injuries, specifically traumatic eye injury from penetrating wounds and traumatic brain injury-related visual disorders, rank second only to hearing loss as the most common injury among active military.[4] In contrast to the 80% return-to-duty rate for most battle trauma injuries, only 20% of these eye-injured warfighters return to duty.[4]

The standard treatment for abrasions is the application of an ophthalmic antibiotic ointment and patching of the affected eye(s) for 24 hours or longer. This therefore causes an inevitable and unpredictable period of loss of full function of the soldier. Current treatments for penetrating injuries rely on use of donor amniotic membrane, which is expensive, scarce in field hospitals, difficult to work with surgically, and can lead to host rejection. The development of a biomaterial that can repair multiple layers of the cornea, treat corneal scarring, or even repair full thickness injuries would be a significant advance in restoring native tissue architecture and function. An implantable EMERGE biocomposite would accelerate wound healing, ensure a faster return to full function, and improve the quality of life for those inflicted with eye injuries.

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[2]B. D. Owens, J. F. Kragh, Jr., J. C. Wenke, J. Macaitis, C. E. Wade, and J. B. Holcomb, *The Journal of trauma*, 2008, **64**(2), 295-299.
[3]D. Tong and R. Beirne, *Military Medicine*, 2013, **178**(4), 421-426.
[4]Anon. 'National Alliance for Eye and Vision Research',[viewed January 20, 2014]; Available from: http://www.eyeresearch.org/pdf/internationalinnovatons.pdf.

Additionally, this EMERGE biocomposite could be used as a single, point-of-care therapeutic material, with integrated pharmaceutical delivery and healing benefit, enabling first responders to effectively treat casualties on the battlefield as close to time of injury as possible. Sterile, dehydrated collagen implant packs with long shelf life could be available in field hospitals for almost immediate surgery.

5. Changes/problems

There have been no major changes to the Statement of Work or to our approach.

Minor changes to details in outlined experiments have been made, listed here:

- Due to high performance of collagen membrane alone, collagen electrospun fibers do not need to be included in the collagen membranes, as originally suggested in the SOW. (Aim 2)
- Optimized nanocarriers for the bioelectric modulators have been identified as PLGA nanoparticles, an option not explicitly called out in the SOW. (Aim 3)
- We have chosen to continue studies using a rat cornea instead of a human cadaver eye. Rat cornea wounds have a larger wound electric field and they are more readily available.

None of these changes are expected to affect timeline, budget, or spirit of the project plan in any way.

6. Products

Publications

To date, we have not published work done in EMERGE in scientific journals. We plan to publish first results from EMERGE in year 3 of the project.

Technologies and Techniques

Work in this project is contributing to the development of new techniques. We have developed protocols to synthesize strong, transparent, collagen membranes using a unique process involving the influence of electric field. We have also developed aminophylline-loaded PLGA particles. For both of these techniques, internal IP disclosures have been filed. The IP disclosures, listed below, have been filed with iEdison.

Titles of submitted IP Disclosures:

“Aligned collagen materials with drug delivery capabilities for enhanced wound regeneration”

"Aminophylline loaded poly(lacto-co-glycolic acid) nanoparticles for ocular wound healing."

7. Participants and other collaborating organizations.

Personnel	**Role**	**Approximate Effort**
JHU/APL		
Morgana Trexler	Principal Investigator	35 hrs
Leslie Hamilton	Materials Scientist, Project Manager	143 hrs
Xiomara Calderón-Colón	Materials Scientist, Collagen materials synthesis, drug delivery	400 hrs
Lance Baird	Chemist, Nanoparticle synthesis, Drug delivery	241 hrs
Melanie Morris	Materials Scientist	111 hrs
Cavin Mooers	Electron Microscopy Technician	34 hrs
Various	Program Management	43 hrs
UC Davis		
Min Zhao	Co-PI	210 hours
Brian Reid	Scientist, Electrophysiology	1,518 hours
Volodymyr Ryzhuk	Scientist	70 hours
Li Ma	Scientist	140 hours

8. Special Reporting Requirements

EMERGE –

Engineered Materials that create Environments for ReGeneration via Electric Field

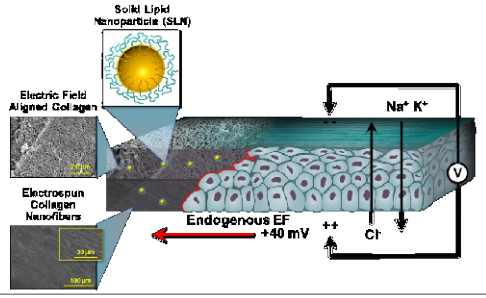
DUNS: 00-191-0777, EIN: 52-0595110, Log Number: MR130110

PI: Morgan Trexler, Ph.D. Org: Johns Hopkins University Applied Physics Laboratory
Co-PI: Min Zhao, M.D., Ph.D. Org: UC Davis Proposed Award Amount: $1M

Study Goal: To develop an ocular implant material with biomimetic chemistry and nanotopography and unique drug delivery functionality to 1) provide biochemical and biophysical cues for enhanced cell migration, differentiation and proliferation and 2) deliver wound electric field enhancing pharmaceuticals for enhanced bioelectronics cues.

Approach

1. Correlate the presence and dosage of the bioelectric modulators with wound healing.
2. Develop a collagen-collagen biocomposite with tailorable nanostructure and collagen fibril alignment, and study the relationship between collagen fiber topography and wound healing to enable optimization of the biocomposite structure.
3. Incorporate bioelectric modulators into the collagen-collagen composite and characterize release.
4. Measure endogenous EF at corneal wound sites and correlate with effects of bioelectric modulators, pharmacological delivery system, and collagen biocomposite to assess efficacy of these materials and pharmaceuticals for healing.



A collagen biocomposite will accelerate ocular wound healing via multiple wound healing strategies: biochemical cues, topographical cues and delivery of bioelectric modulators that enhance the wound EF.

Timeline and Cost

Aims	FY15	FY16	FY17
Correlation of bioelectric modulators to wound EF, healing			
Development of collagen biocomposite			
Encapsulation and release of bioelectric modulators			
Optimization of materials to maximize therapeutic benefit			
Cost	$316	$345	$339

Updated: 06 February 2014

Goals/Milestones

FY15 Goals – Selection of bioelectric modulators. Collagen biocomposite synthesis.
- ❑ Screening of pharmacological agents using rat corneal model (RCM) and human cornea test platform (HCTP)
- ❑ Alignment of collagen fibrils, fabrication of high strength gels

FY16 Goal – Correlation of topography effects on EF and wound healing. Engineering of tailored release of bioelectric modulators.
- ❑ Analysis using vibrating probe technique (RCM, HCTP)
- ❑ Characterization of release of agents from nanoparticles/fibers

FY17 Goal – Demonstration of efficacy of biomaterial-pharmacological agent combination for EF stimulation and wound healing.
- ❑ Optimization of collagen biocomposite topography and bioelectric agent delivery
- ❑ Quantification of effects on EF and healing (RCM, HCTP)