

AWARD NUMBER: W81XWH-16-2-0067

TITLE: Extremity Regeneration of Soft Tissue Injury Using Growth Factor-Impregnated Gels

PRINCIPAL INVESTIGATOR: Simon Talbot, MD

CONTRACTING ORGANIZATION: Brigham and Women's Hospital, Boston, MA

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT All animal experiments have been completed. A second no cost extension has been approved in order to complete the remaining toxicology testing with CRO, Toxikon. These studies were delayed due to the COVID19 pandemic. All other experiments to evaluate the dose-response relationship, determine function of growth factor on nerve regeneration and function of each growth factor on independent nerve versus ischemic injuries have been completed. The Wyss Institute has completed work on the process and development of the alginate gels and growth factors.					
15. SUBJECT TERMS Nerve and vessel regeneration. Growth factor: VEGF and IGF.					
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1. INTRODUCTION:

The overarching, long-term goal of this project is to develop technologies that maximize restoration of severely injured limbs by restoring muscle and nerve functions and avoiding amputation. This research specifically focuses on promoting regeneration of the injured host tissue by use of exogenous growth factors. A natural soft polymer gel material, alginate, has been fabricated to release two natural growth factors – vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1). Repeated injections of growth factor-alginate material are performed following a surgically induced traumatic ischemic injury and followed with muscle biopsies and nerve conduction studies to track regeneration. Preliminary results from small animal studies show that this approach can promote expansion of the host cells, and enhance restoration of blood flow, regeneration of muscle tissue, and reconnection of nerves. Currently, this project is being tested in a large animal swine model for its effectiveness in restoring blood flow, muscle and nerve tissue, and connection of nerve to muscles. The project will extend development of the injectable gel into a prototype product, suitable for commercialization.

KEYWORDS:

Vascularized endothelial growth factor (VEGF)
Insulin-like growth factor-1 (IGF-1)
Alginate gel
Ischemia-reperfusion
Large animal model

2. ACCOMPLISHMENTS:

What were the major goals of the project?

Subtask	Timeline in Months	Completion
Subtask 1.1: Submission of IACUC protocol for Aims 1 and 2	1-4	Completed
Subtask 1.2: Process and method development product	1-8	Completed
Subtask 1.3: Development of a large animal model	4-8	Completed
Subtask 1.4: Development of assays (histology), functional studies (walking) and electrophysiology studies (EMG/NCS)	4-8	Completed
Subtask 1.5: Evaluation of dose-response relationship in limb transection model	8-12	Completed
Subtask 2.1: Determine function of each of VEGF and VEGF+IGF1 on nerve regeneration	12-18	Completed
Subtask 2.2: Determine function of each VEGF and VEGF+IGF1 on ischemia-reperfusion	18-24	Completed
Subtask 3.1: Method and process qualification	24-36	90%
Subtask 3.2: GLP pharmacology-toxicity studies	20-24	75% (3 of 4 studies complete)
Subtask 3.1: Determine proposed clinical design	24-25	10%
Subtask 3.2: Determined proposed pharmacology-toxicity study design	24-25	10%

What was accomplished under these goals?

During the fifth year of study, a manuscript describing our experimental large animal study model was published in *Annals of Anatomy: A Yorkshire swine (Sus scrofa domestica) model for nerve regeneration and ischemia based on the sciatic nerve and femoral artery*.

All surgical experiments were completed in 2020. Pathology slides have been transferred from USUHS to BWH and further histopathology evaluation is underway. Ongoing systemic review and analysis of all data has been delayed due to travel restrictions and access to the USUHS laboratories.

Through a contracting research laboratory, Toxikon, a 28-day repeated dose toxicity study in rats is scheduled to begin in March, 2022. The COVID-19 pandemic has continued to delay this final toxicology study and a no-cost extension has been approved until September of 2022 to ensure completion.

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

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

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

During the final years of this project, we will complete analysis of the muscle biopsies and histopathology en bloc. We will complete the final GLP toxicity study with the 28-day repeated dose toxicity study in rats. We will review our data and consider pre-IND.

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

A no-cost extension has been approved to continue work until September of 2022.

Actual or anticipated problems or delays and actions or plans to resolve them

Our final toxicology study, 28-Day Repeat Dose Toxicity Study in Sprague Dawley Rats with 28-Day Recovery, will begin March of 2022. A no-cost extension has been approved until September of 2022 allowing the completion of this work.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

Significant changes in use or care of vertebrate animals.

Several swine have developed insensate limb wound necrosis ultimately resulting in euthanasia. Through discussions with IACUC and veterinary staff, modifications to post-operative care have been instituted. This includes a modified dressing on the insensate limb and padded flooring to minimize traumatic wounds. Additional support staff will be present to monitor swine during the early post-operative period.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Publication of manuscript:
Kinsley SE, Fernicola SD, Dingle ME, Williams MS, Richardson JM, Taylor D, de Vasconcellos JF, Malone TR, Blattner MR, Smith JK, Oliver A, Koch AL, Riddle LE, Reiter C, Culp WE, Caterson EJ, Nesti LJ, Talbot SG. A Yorkshire swine (*Sus scrofa domestica*) model for nerve regeneration and ischemia based on the sciatic nerve and femoral artery. *Ann Anat.* 2020 Sep 28:151587. doi: 10.1016/j.aanat.2020.151587.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Simon Talbot

Project Role: Principal Investigator

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 6.0

Contribution to Project: Directs and oversees all phases of the study.

Name: EJ Caterson

Project Role: Co-Investigator

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked:

Contribution to Project: Assistance with planning and surgical aspects of the study.

Name: Sarah Kinsley

Project Role: Research Assistant

Research Identifier:

Nearest person month worked: 3.0

Contribution to Project: Involved in coordination and ensuring each phase of the project remains on schedule, writing protocols, purchasing.

Name: David Mooney

Project Role: Co-Principal Investigator

Research Identifier:

Nearest person month worked:

Contribution to Project: Involved in management of Wyss Institute input to project.

Name: Ed Doherty

Project Role: Co-Principal Investigator

Research Identifier:

Nearest person month worked:

Contribution to Project: Involved in coordination of production of Wyss gels.

Name: Alexander Stafford

Project Role: Scientist

Research Identifier:

Nearest person month worked:

Contribution to Project: Involved in production of gels.

Name: Des White
Project Role: Research Associate
Research Identifier:
Nearest person month worked:
Contribution to Project: Involved in production of gels.

Name: Tracy Snyder
Project Role: Research Associate
Research Identifier:
Nearest person month worked:
Contribution to Project: Involved in production of gels.

Name: Leon Nesti
Project Role: Co-Principal Investigator
Research Identifier:
Nearest person month worked:
Contribution to Project: Involved in management of USUHS staff and laboratory including coordination of animal experimentation on site.

Name: Denis Taylor
Project Role: Research technician
Research Identifier:
Nearest person month worked:
Contribution to Project: Involved in day-to-day running and local coordination of activities.

Name: Jaira Vasconcellos
Project Role: Staff scientist
Research Identifier:
Nearest person month worked:
Contribution to Project: Involved in day-to-day running and local coordination of activities.

Name: Amal Nadel
Project Role: Program manager
Research Identifier:
Nearest person month worked:
Contribution to Project: Involved in coordination of activities through USUHS.

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: United States Uniformed Health Services

Location of Organization: Associated with Walter Reed Military Medical Center in Bethesda, MD

Partner's Contribution to the project: Facilities and collaboration

Organization name: Wyss Institute for Biologically Inspired Engineering

Location of Organization: Associated with Harvard University. Located in Boston, MA

Partner's Contribution to the project: Collaboration and in-kind support developing the alginate and growth factor.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

Extremity Regeneration of Soft Tissue Injury Using Growth Factor Impregnated Gels

Log Number: DM153165

Award Number: W81XWH-16-2-0067

PI: Simon G. Talbot, MD

Org: Brigham and Women's Hospital

Award Amount: \$2.1 M



Study/Product Aim(s)

Hypothesis: Injection of growth factor impregnated hydrogels can restore blood flow, promote muscle and nerve regeneration, and restore nerve connections to muscle.

- Aims: Evaluate biocompatibility and efficacy of alginate gel-based delivery of VEGF and IGF-1 in a large animal model of limb injury including ischemia-reperfusion and nerve transection-repair in support of future human clinical studies.

Approach

Experiment 1: Determine optimal ischemia time and optimal growth factor dose in large animal model.

Experiment 2: Determine effect of VEGF and IGF-1 on nerve regeneration.

Experiment 3: Determine effect of VEGF and IGF-1 on ischemia-reperfusion.



A Yorkshire swine (*Sus scrofa domestica*) model for nerve regeneration and ischemia based on the sciatic nerve and femoral artery. Kinsley SE, Femicola SD, Dingle ME, Williams MS, Richardson JM, Taylor D, de Vasconcellos JF, Malone TR, Blattner MR, Smith JK, Oliver A, Koch AL, Riddle LE, Reiter C, Culp WE, Catterson EJ, Nestli LJ, Talbot SG. *Ann Anat.* 2020 Sep 28:151587.

Timeline and Cost

Activities	2017	2018	2019
Determine optimal ischemia time and optimal growth factor dose in large animal model.	~100	0	0
Determine effect of VEGF and IGF-1 growth factor on nerve regeneration.	0	~100	0
Determine effect of VEGF and IGF-1 growth factor on ischemia-reperfusion.	0	0	~100
Estimated Budget (\$K)	733	702	665

Updated: 10/22/2021

Goals/Milestones

CY17 Goal – Determine optimal ischemia time and growth factor dose

- Develop large animal model
- Submit to IACUC and ACURO
- Begin experiment 1 on 20 animals
- Modify IACUC protocol to minimize postoperative risks
- Preliminary testing of alginate gel confirms consistency of product

CY18 Goal – Determine effect of growth factor on nerve regeneration

- Complete experiments to determine ischemia time
- Begin dose experiments on 15 animals

CY 19 Goal – Determine effect of growth factor on ischemia-reperfusion

- Experiment 3
- Initiate development of IND application to FDA

Comments/Challenges/Issues/Concerns

- Approved no-cost extension in 2021 to complete Toxikon toxicology studies
- Delays due to COVID-19

Budget Expenditure to Date

Actual Expenditure: \$1,888,690.58 (direct costs: \$1,531,673.46)

9. APPENDICES:

- Publication