AWARD NUMBER: W81XWH-17-1-0059

TITLE: Large Extremity Peripheral Nerve Repair in Nonhuman Primate Models

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CONTRACTING ORGANIZATION: Massachusetts General Hospital
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REPORT DATE: April 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE		Form Approved OMB No. 0704-0188
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**		
1. REPORT DATE April 2019	**2. REPORT TYPE** Annual	**3. DATES COVERED** 15 Mar 2018 - 14 Mar 2019
4. TITLE AND SUBTITLE Large Extremity Peripheral Nerve Repair in Nonhuman Primate Models		**5a. CONTRACT NUMBER**
		5a. GRANT NUMBER W81XWH-17-1-0059
		5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S) Robert W. Redmond Ph.D. E-Mail: redmond@helix.mgh.harvard.edu		**5d. PROJECT NUMBER** **5e. TASK NUMBER** **5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES) The Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114.		**8. PERFORMING ORGANIZATION REPORT NUMBER**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSOR/MONITOR'S ACRONYM(S) 11. SPONSOR/MONITOR'S REPORT NUMBER(S)
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited		
13. SUPPLEMENTARY NOTES		

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14. ABSTRACT

The overarching hypothesis of this proposal is that a rapid, simple, light-activated sealing technology can provide a more secure wound closure and reduce complications leading to improved outcomes for wounded warfighters following traumatic penetrating colon injury. Penetrating bowel wounds can be rapidly sealed and stabilized using biocompatible patches in conjunction with light-activated bonding. Our objective is to determine the optimal implementation strategy for this technology in a large animal model that recapitulates the military trauma scenario and to address a priority research area in the Combat Casualty Care Research Program "to identify and develop medical techniques and materiel for early intervention in life-threatening battle injuries".

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The aim of this JWMRP proposal is to employ a relevant large animal model of peripheral nerve injury involving large segmental deficit in the upper limbs that recapitulates human anatomy and is capable of objective functional outcome testing that is not possible in other large animal models. With this model we will determine whether our improved method of restoring continuity to peripheral nerves by photosealing a commercial acellular nerve allograft across the deficit can produce outcomes equivalent to standard of care autologous grafting for wounded warfighters that do not have sufficient autologous donor nerve due to extensive combat trauma. Improving functional recovery for wounded warfighters in this manner would have a major impact on quality of life and well-being of such wounded warfighters.

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

Peripheral nerve repair, nerve graft, nerve allograft, non-human primate, tissue bonding, photosealing, rose Bengal, PTB, Avance, functional recovery, EMG, muscle mass retention, PSOCT.

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?
The major goal of this project is to improve functional outcomes following repair of large nerve deficit injures in wounded warriors that utilize acellular nerve allograft where autologous nerve graft is unavailable following severe trauma.

Milestones for this award are listed below, along with percentage completion to date (in bold0 where appropriate.

A. Regulatory approval (MGH IACUC and ACURO) of injury and repair models in Rhesus monkeys (Months 1-4). **100% complete**
B. Purchase and receipt of laboratory supplies. (Months 1-2) **100% complete**
C. Harvest of human amniotic membrane (HAM) from placenta obtained from MGH under Discarded Tissue Protocol (Months 1-3). **100% complete**
D. Crosslinking of HAM with EDC/NHS to make xHAM (Months 2-4). **100% complete**
E. First meeting of MGH team with surgical advisors at MGH to discuss progress and suggest improvements to the research approach (Month 6). **100% complete**
F. Purchase and acclimatization of twenty Macaque Monkeys (Months 4-8 at 4 per month). **100% complete.**
G. Training of monkeys in behavioral task used to evaluate for functional recovery after repair (Months 4-9). **100% complete**
H. Survival surgeries for peripheral nerve defect and repair in Rhesus monkey model (Months 5-10). **100% complete**
I. Second meeting of MGH team with military surgeons advisors at MGH to discuss progress and suggest improvements to the research approach (Month 12).
J. Serial monthly behavioral testing for recovery of function in monkeys. (Months 5-22) **70% complete**.

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K. Serial electrophysiology measurements in monkeys (Months 5-22) **60% complete**.

L. Third meeting of MGH team with military clinical advisors at MGH to discuss progress and suggest improvements to the research approach (Month 18).

M. Euthanasia of Monkeys at one-year post-operative time points (Months 17-22) **35% complete**.

N. Harvest of nerve repair complex at euthanasia and preparation of samples for histology and histomorphometry (Months 17-22) **35% complete**.

O. Harvest of innervated muscle at euthanasia and determination of retention of muscle mass with respect to unoperated contralateral limb. (Months 17-22) **35% complete**.

P. Analysis of histomorphometric data from nerve cross-sections. (Months 22-24).

Q. Final meeting of MGH team with military surgeons advisors at MGH to discuss results and conclusions form the entire project and plan for immediate human trial, if successful. (Month 24).

R. Preparation of report and manuscript based on outcomes.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

In the second year of this project we have completed all NHP surgeries to create a large deficit in the radial nerve in the upper arm, followed by repair by one of three methods. 1. standard of care microsurgery using reversed autograft (control); 2. Photosealed acellular nerve allograft (PTB/ANA, Figure 1); 3. Microsurgical attachment of ANA.

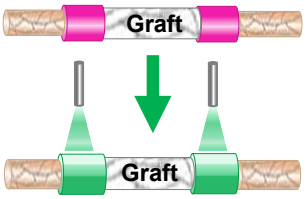

***Figure 1**: Schematic of photosealing approach to neurorraphy by applying the photoactive dye (pink) rose Bengal to crosslinked amnion and wrapping around coaptation sites of ANA, followed by illumination with green light to seal the graft in place.*

All NHPs have undergone serial electrophysiological testing at least out to 4 months and most have been tested at 8 months. Six NHPs have completed the 12-month study and have been euthanized with tissue (muscle and nerve) collection for analysis of muscle mass and histomorphometry. The respective tissues on the unoperated arm have also been collected for comparative purposes. The operative schedules are shown below with completed tasks in bold font.

Table 1: Operative Schedules for all NHPs in the Study

NHP #	DOB	Group	OR Date	Euthanasia Date
8117	4/17/13	Control	**1/8/2018**	**1/14/19**

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8017	5/12/13	Control	**1/19/2018**	**1/22/19**
7817	3/25/13	PTB	**2/5/2018**	**2/4/19**
7917	3/16/13	PTB	**2/13/2018**	**2/11/19**
8217	3/19/13	Control	**3/14/2018**	**3/15/19**
8317	5/6/13	Control	**3/21/2018**	**3/21/19**
1118	4/11/11	PTB	**6/11/2018**	5/6/19
1218	3/9/11	Avance/Suture	**6/12/2018**	5/24/19
818	6/25/11	Avance/Suture	**6/18/2018**	6/3/19
1018	8/3/11	Avance/Suture	**6/19/2018**	6/10/19
918	6/10/11	PTB	**6/25/2018**	5/23/19
1318	8/26/11	PTB	**6/26/2018**	6/17/19
1918	4/25/11	Control	**8/6/2018**	8/5/19
2118	2/1/12	Avance/Suture	**8/8/2018**	8/12/19
1418	6/16/11	Control	**8/13/2018**	8/19/19
1718	11/25/11	PTB	**8/14/2018**	8/26/19
1518	5/23/11	PTB	**9/17/2018**	9/17/19
2018	4/10/11	Avance/Suture	**9/25/2018**	9/25/19

Functional recovery was performed as follows: Prior to each radial nerve surgery (Figure 2A) the NHPs were acclimatized to their environment and underwent repeated training to reach out the cage through a cylindrical tube and obtain a food treat from a platform that could be adjusted in height Figure 2B). Resection of the nerve segment causes loss of extension and 'wrist drop', which recovers over time if nerve regeneration occurs. Video recording allowed freeze frame analysis of the wrist extension angle at the maximum platform height that could be reached by the NHP (Figure 2C).

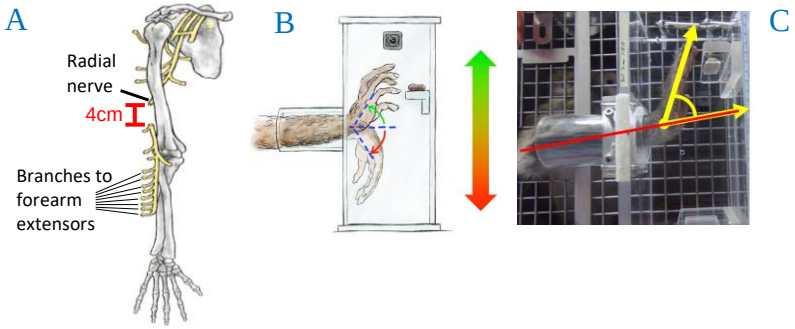

Figure 2: *NHP model for nerve deficit and recovery. (A) a 4 cm radial nerve deficit is surgically created to induce loss of wrist extension. (B) Functional recovery is measured by a behavioral test that measures the*

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extent the NHP can extend the wrist to retrieve a food treat form a platform that is adjustable in height. (C) Photo showing evaluation of wrist extension angle from freeze-frame video analysis.

Functional recovery in each NHP was measured each month and the results to date are shown in Figure 3. Although we are at an early stage it would appear that results are binary in nature. We either see total recovery or no recovery at all. Each repair group shows examples of each behavior. An additional observation is a reduction in the rate of ANA repairs with respect to autograft repair. This is to be expected due to lack of initial Schwann cell support and existing microvasculature in the ANA, both of which contribute considerably to the regenerative response.

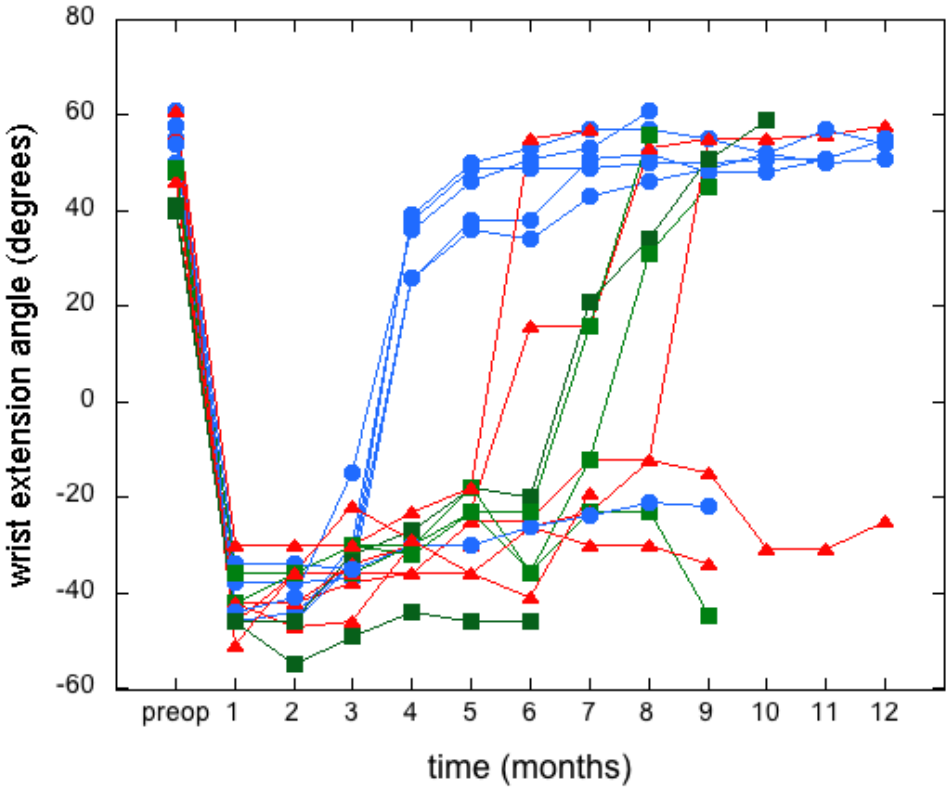

***Figure 3**: Serial functional recovery measurements for each NHP as a function of time and repair method. (● - control, ▲ - PTB/ANA, ■ - suture/ANA).*

For animals that have reached the end of the 12-month study we have measured the innervated extensor muscle mass for the operative and non-operative arms for each NHP. The results, expressed as percentage change from the non-operated side, are shown in Figure 4 and compared to the final wrist extension capability for each

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animal. We appear to have good cross-correlation between outcome metrics as a lack of functional recovery correlates well with muscle wastage in NHP #7817.

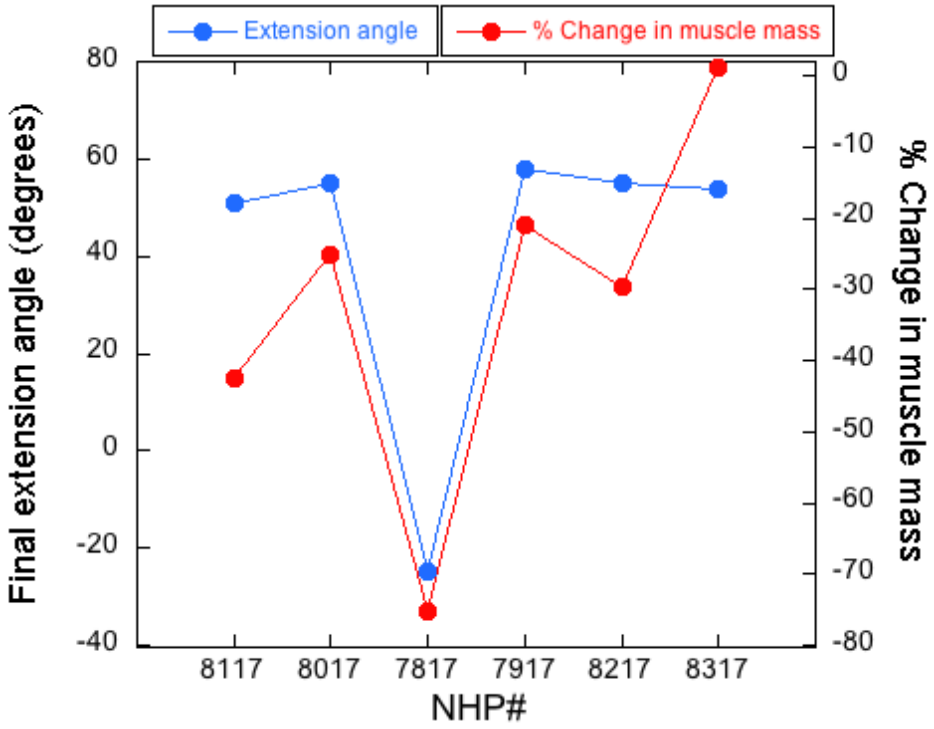

Figure 4: *Comparison of functional recovery and muscle mass retention data for animals reaching study end point of 12 months.*

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

Dr. Hansdorfer has received advanced microsurgery training from Dr. Winograd and Dr. Randolph including operating under magnification. His mentored training within the Division of Plastic Surgery at MGH has resulted in accelerate acceptance into a Plastic Surgery Residency Program at Rush University in. Chicago, IL, which he will commence in July 2019. He has also been very active in disseminating the findings of the research at National and International meetings (*vide infra*).

How were the results disseminated to communities of interest?

Results were disseminated at national and international conferences (including MHSRS 2018) , through publications and abstracts, as described in a following section.

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

In the next reporting period we will conclude all outcomes measurements for every NHP, with the final euthanasia planned for September 2019. Following the end of study for each animal we will correlate functional outcome with muscle mass retention, electrophysiology and histomorphometry to provide an in-depth analysis of nerve regeneration in each animal and study group in terms of rate and magnitude of regeneration. We have already drafted a manuscript on the behavioral model and will then follow up with a paper that will evaluate nerve regeneration as a function of the approaches used and how these may be improved in the future.

4. IMPACT*:*

We firmly believe that our NHP model is by far the best model in current use for clinically-relevant evaluation of any intervention (surgical, pharmacological or rehab) targeted to improving nerve regeneration and functional recovery in patients. In addition the use of photosealing of ANA has been shown to be capable of functional recovery equivalent to standard of care autograft in some cases that extends hope to victims of polytrauma, such as wounded warriors, that have no autologous nerve available for grafting.

What was the impact on other disciplines?

The photosealing approach has a wide capability and is currently being evaluated for sealing of penetrating bowel injury and prevention of post-surgical leaks and adhesions and will be explored in the near future as an augmentation for orthopedic repair of tendon, ligament and cartilage injuries.

What was the impact on technology transfer?

The technology behind the photosealing of nerve grafts was already protected and patented prior to the start of this study. We are very close to launching a start-up company in the vascular repair space and expect nerve repair to be one of the next indications to be commercialized.

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
MGH ran into a problem of shortage of large animal housing that delayed the initiation of the NHP surgeries, resulting in a request for a no-cost extension until 3/1/2020. Since that time the housing shortage has been alleviated and all surgeries have been performed and the study will easily be concluded with the NCE time-frame.

Changes that had a significant impact on expenditures
None

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Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
None

Significant changes in use or care of human subjects
N/A

Significant changes in use or care of vertebrate animals
None

Significant changes in use of biohazards and/or select agents
None

Institutional IACUC and ACURO Approvals

- 12/28/2016: Submission of protocol to MGH IACUC (#2016N000627)
- 3/23/2107: Approval of protocol by MGH IACUC
- 3/31/2017: Submission of protocol to ACURO
- 6/26/2017: Approval of protocol by ACURO
- 10/10/2017: Submission of amended protocol to MGH IACUC
- 11/06/2017 Approval of amended protocol by MGH IACUC
- 11/17/2017: Submission of amended protocol to ACURO
- 12/4/2017: Approval of amended protocol by ACURO

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

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

Journal publications.

Medical Applications of Rose Bengal- and Riboflavin-Photosensitized Protein Crosslinking. Redmond RW, Kochevar IE. *Photochem Photobiol*. 2019. doi: 10.1111/php.13126. [Epub ahead of print] PMID: 31111489.

Invited Talks:

Potential Clinical Applications of Protein Photocrosslinking. Redmond RW. *Annual Meeting of the American Society for Photobiology*, April 2018, Tampa, Florida.

Large Gap Peripheral Nerve Repair in a Non-Human Primate Model Utilizing Photochemical Tissue Bonding (PTB). Hansdorfer MA, Tsui JT, Visaggio MC, Runyan GG, Randolph MA, David WS, Winograd JM, Redmond, RW. Presented at 29th *Annual Richard J. Smith Conference*. 2018 April 27; Boston, MA (**Accepted**).

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A Clinically Relevant Animal Model to Quantitatively Evaluate Functional Outcomes of Large-Gap Peripheral Nerve Repair. Hansdorfer MA, Tsui JT, Visaggio MC, Runyan GG, Randolph MA, David WS, Winograd JM, Redmond, RW. Accepted for Presentation at *Massachusetts General Hospital Clinical Research Day*. 2018 October 4; Boston, MA

Preclinical Studies of Photocrosslinking Technologies for Tissue Repair and Regeneration. Redmond RW. *17th International Congress on Photobiology*, 2019, August 21, Barcelona, Spain. (**Accepted**).

Conference Proceedings:

Hansdorfer MA, Tsui JM, Visaggio M, Runyan GG, Zarfos SD. Randolph MA, Redmond, RW. ***Light-Activated Sealing to Improve Outcomes Following Penetrating Bowel Trauma***. Harvard Research Day. 2019 March 9; Boston, MA/

Hansdorfer MA, Tsui JM, Visaggio M, Runyan GG, Randolph MA, David WS, See RS, Valerio IL, Winograd JM, Redmond, RW. ***Large Gap Peripheral Nerve Repair in a Non-Human Primate Model: Improving Outcomes Utilizing Photochemical Tissue Bonding (PTB) with Acellular Nerve Allograft (ANA)***. 64th Annual Plastic Surgery Research Council (PSRC) Meeting. 2019 May 2-5; Baltimore, MD (**Accepted**).

Hansdorfer MA, Tsui JM, Visaggio M, Runyan GG, Randolph MA, David WS, See RS, Valerio IL, Redmond, RW Winograd JM. ***Improving Outcomes of Large Gap Peripheral Nerve Repair with Photochemical Tissue Bonding (PTB) and Acellular Nerve Allograft (ANA)*.** Accepted for Presentation at the 60th Annual New England Society of Plastic and Reconstructive Surgeons. 2019 May 31-June 2; Newport, RI. (**Accepted**).

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)**

- **Technologies or techniques**

Nothing to report

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

Nothing to report

- **Other Products**

We have developed a new non-human primate model for the study of any intervention (surgical, pharmacological or rehab) that is far better than any current animal model for the study of nerve regeneration in a clinically-relevant manner. We expect this model to be the standard in future translational peripheral nerve regeneration studies. This model will be published in full in the coming months.

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7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: *Robert W. Redmond PhD*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *2*
Contribution to Project: *Dr. Redmond is responsible for overall coordination of the project*

Name: *Mark A. Randolph MAS*
Project Role: *Investigator*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*
Contribution to Project: *Mr. Randolph has been instrumental in designing animal protocols and in the behavioral testing design.*

Name: *Marek Hansdorfer MD*
Project Role: *Research Fellow*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *6*
Contribution to Project: *Dr. Hansdorfer has been the lead Fellow on this project and has been involved in all day-to day aspects of regulatory approvals, experimental planning, surgical training and behavioral testing design.*

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?

Nothing to Report.

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