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TITLE: Development of a Supercooled Limb Preservation protocol

PRINCIPAL INVESTIGATOR: Korkut Uygun, PhD

CONTRACTING ORGANIZATION: Massachusetts General Hospital
Boston MA 02114

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
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Massachusetts General
Hospital
55 Fruit St Boston MA 02114

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14. ABSTRACT

The project aims to develop a novel technology to preserve vascular composite allografts for extended periods. This project uses a rat limb transplant model and aims to preserve grafts for up to 3 days.

In the second year, we developed a supercooled VCA preservation modality and tested by tissue energy charge. We are finishing testing the duration limits of this protocol. Year 3 will focus on transplant testing of the protocol.

15. SUBJECT TERMS
Organ Preservation, VCA transplantation, limb transplantation, supercooled storage

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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

Static cold storage (SCS) of allografts with University of Wisconsin (UW) solution at 4 °C is the current clinical standard in transplantation, but is limited to few hours for vascular composite allografts (VCAs). In our prior studies, our group develop a subzero nonfreezing (SZNF) preservation approach to extend storage of rat livers up to 3 days. In this project, we aim to develop a similar SZNF preservation approach for rat limbs and demonstrate viability after 3 days storage with transplantation.

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

Organ Preservation, VCA transplantation, limb transplantation, supercooled storage

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

1. Milestone #1 ACURO approval obtained. (Months 1-5) **100% complete**
2. Milestone #2 Complete evaluation of Machine perfusion on VCA viability. (Months 5-24) **90% complete**
3. Milestone #3 Establish a SZNF protocol for VCA tissues (Endpoint: Tissue Energy Charge). (Months 12-15). **100% Complete.**
4. Milestone #4 Identify limits of SZNF for rat limbs ((Endpoint: >80% transplant survival). (Months 12-36) **25% Complete.**

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.

There were two major accomplishments in this report year as indicated in the milestones list. Briefly, we have tested the viability of perfused rat limbs by transplantation and demonstrated improved success compared to static cold storage at six hours of storage. We also developed a supercooled limb allograft preservation protocol, and tested viability with tissue energy charge.

Below we elaborate on the perfusion protocol, transplant results, and describe the supercooling protocol developed.

Ex Vivo Rat Limb Perfusion

As a results of studies in years 1 and 2, we developed a perfusion protocol which allows for viable preservation of rat hindlimbs ex vivo, and completed testing for two durations (3 and 6 hours), and tested by transplant as discussed in the next section. Figure 1 below describes the initial optimization performed with three alternative perfusion media formulations, and Figure 2 shows histology and apoptosis staining results. Figures 3 and 4 and show detailed results of the 6 hour perfused rat limbs with our final protocol, with results indicating stable perfusion of rat limbs without any indications of hypoxia or injury.

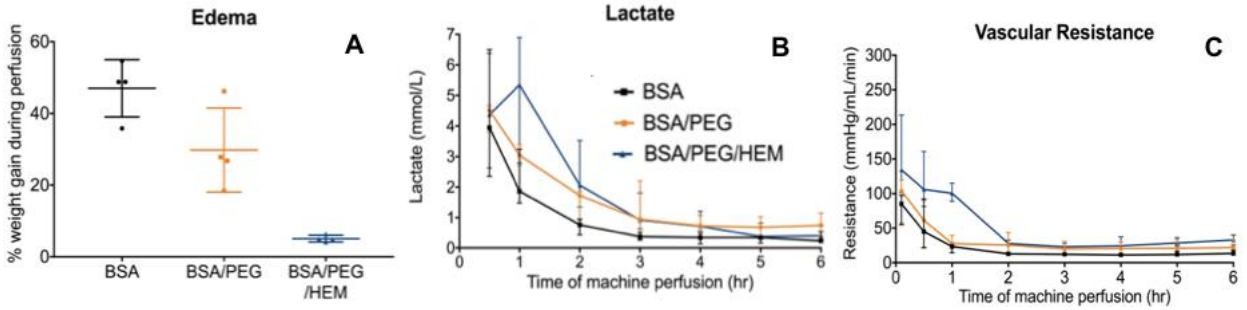

Figure 1: Subnormothermic machine perfusion of rat hind limbs. A) Edema (weight gain during perfusion), least in the hemopure group ($P<0.05$). **B)** Lactate levels drop indicating no sustained hypoxia during perfusion. **C)** Vascular resistance, also dropping then stabilizing during perfusion although fastest with the hemopure group (n=4 per group). All comparisons by ANOVA with significance threshold at $p<0.05$.

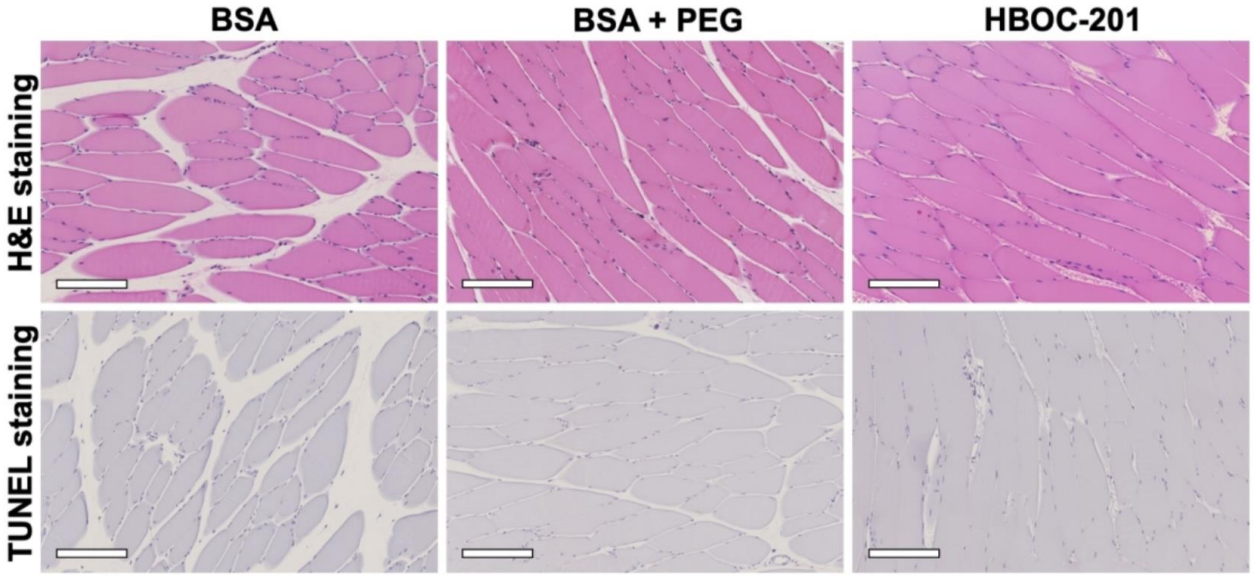

Figure 2. Representative muscle histology of limbs after 6 hours of perfusion with BSA, BSA + PEG and HBOC- 201 respectively. Upper panels represent H&E stained biopsies, lower panels show TUNEL stained biopsies. All biopsies show a normal polygonal structure with no signs of apoptosis. All slides are shown at 10x magnification, and the white box indicates 200 mm. Abbreviations used: H&E = hematoxylin & eosin, BSA = bovine serum albumin, PEG = polyethylene glycol and HBOC-201 = hemoglobin-based oxygen carrier – 201.

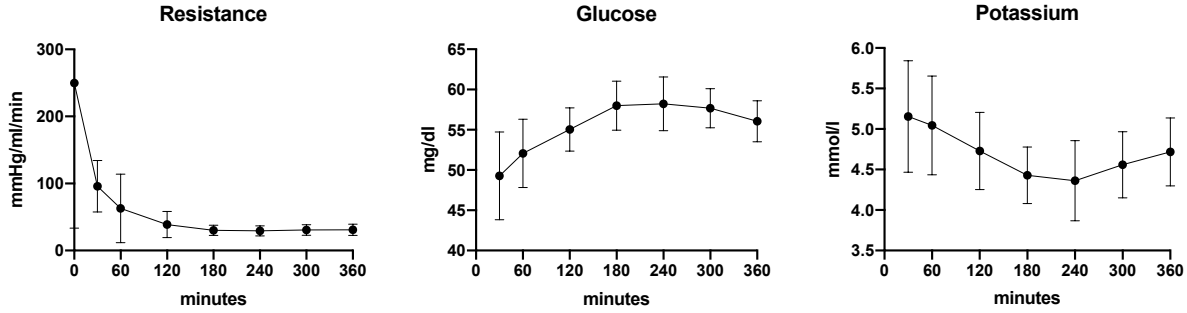

Figure 3. Profiles of vascular resistance, glucose and potassium during 6 hour machine perfusion. Overall the grafts show low resistance after an initial adjustment period, stable potassium levels indicated no cellular injury during perfusion, and stable glucose levels indicating stable metabolic activity.

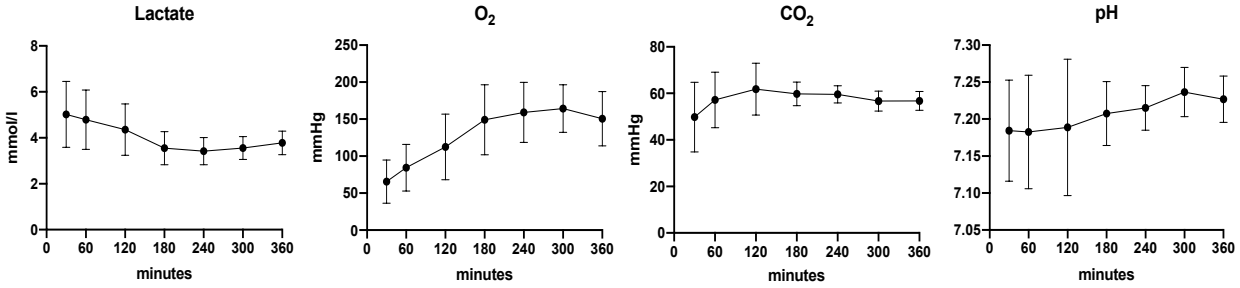

Figure 4. Profiles of lactate, oxygen, carbon dioxide and pH during 6 hour machine perfusion. As can be observed from stable lactate levels and confirmed by other parameters the limbs are not in a hypoxic state. Note that muscle tissue does clear lactate end therefore the initial lactate levels in the media are not expected to decrease even when the graft is well oxygenated.

Successful Transplantation of Perfused Rat hindlimbs

As of this report we performed 54 of the 60 planned transplantations. As noted in the sections below and also detailed in quarterly reports, we encountered difficulties with the model due to the very small size of blood vessels. After multiple iterations we were able to resolve the problems, with the caveat that in the rat model the limbs are much more prone to ischemic injury compared to livers, and the blood vessels are very prone to injury during flush, and therefore rate of surgical errors (typically revealing as anastomoses failures) are significantly higher compared to for instance rat liver transplants.

As shown below in Figure 5, the results indicate that in our hands rat hind limbs have significantly reduced viability at 6 hours of traditional cold storage (2/5 survival at 30 days post-operative day) and are completely unusable at 24 hours of static cold storage (0/5 survival, as compared with 3/3 for fresh grafts). By comparison, we observed 6/6 survival for 3 hour perfused grafts, and 7/8 survival in 6 hour perfused grafts which is statistically better than 6 hour static cold stored limbs ($p=0.042$ with log-rank test). Figure 6 depicts a 6 hour perfused graft after transplantation and recovery of the animal afterwards. Overall, we therefore conclude that we successfully demonstrated a superior storage modality in this model. Current work involves testing the perfusion model with one more storage duration (24 hours planned), after which we'll conclude work on this milestone.

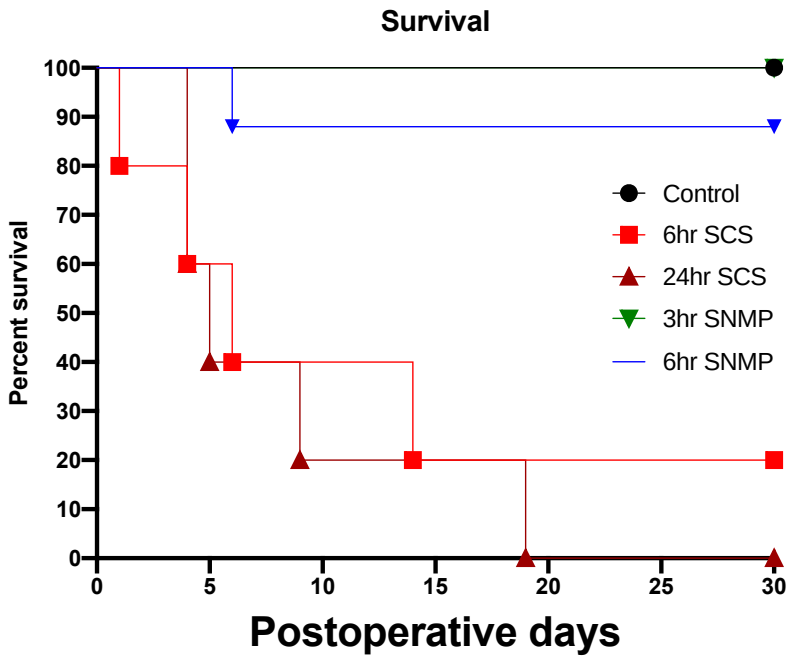

Figure 5: Transplant results of tested preservation protocols and durations. Perfusion preservation is superior to SCS at 6hrs ($p<0.05$).

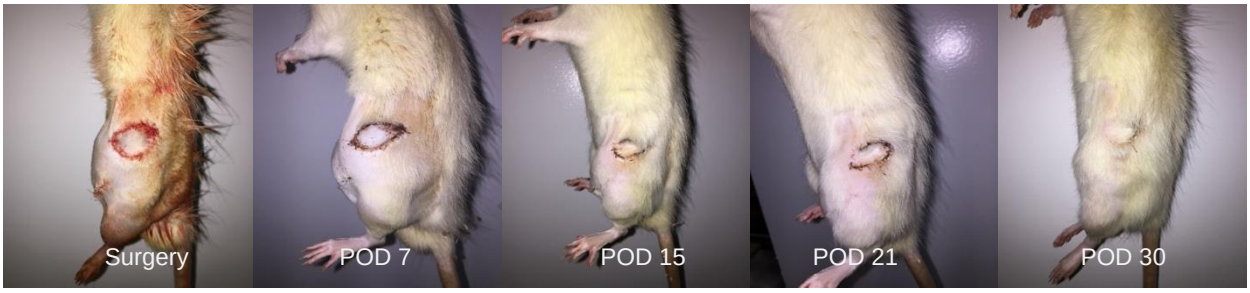

Figure 6: Successful transplant of a 6 hour perfused rat limb graft, and recovery of the recipient animal after graft implantation.

Successful Supercooling of Rat Limbs

We were able to preserve rat limbs that were supercooled for 24hrs in a viable state (**Fig. 7**). Our method relies on i) the use of subzero temperatures (-5°C) to slow metabolism during static storage while avoiding freezing injury, ii) two preservative agents (Polyethylene glycol- PEG and 3-O-Methyl Glucose - 3OMG) to supplement UW solution, and iii) use of machine perfusion for both introduction of these cryopreservation agents, and rewarming to prepare the tissue transplantation, which we have shown in other models to ameliorate some of the cold ischemic injury. While the method is developed based on our prior liver work, it has several modifications including i) use of a different perfusion media designed for muscle tissue and tuned for resolving the edema that is problematic in rat limbs by using a high osmolarity; ii) use of a lower viscosity storage media (HTK) which is critical to avoid shear injury to the endothelial cells during flush before and after supercooling, iii) a specifically tuned flush protocol that minimizes the volumetric flow rate as well as the total media used for flush to avoid injury to the endothelium. As noted below, based on these differences we recently filed an invention disclosure for subzero storage of

vascularized composite allografts. **Figure 8** depicts some parts of the rat limb supercooled storage process. Our plan for year 3 is extending supercooling to 3 days, and testing graft viability by transplantation for select durations.

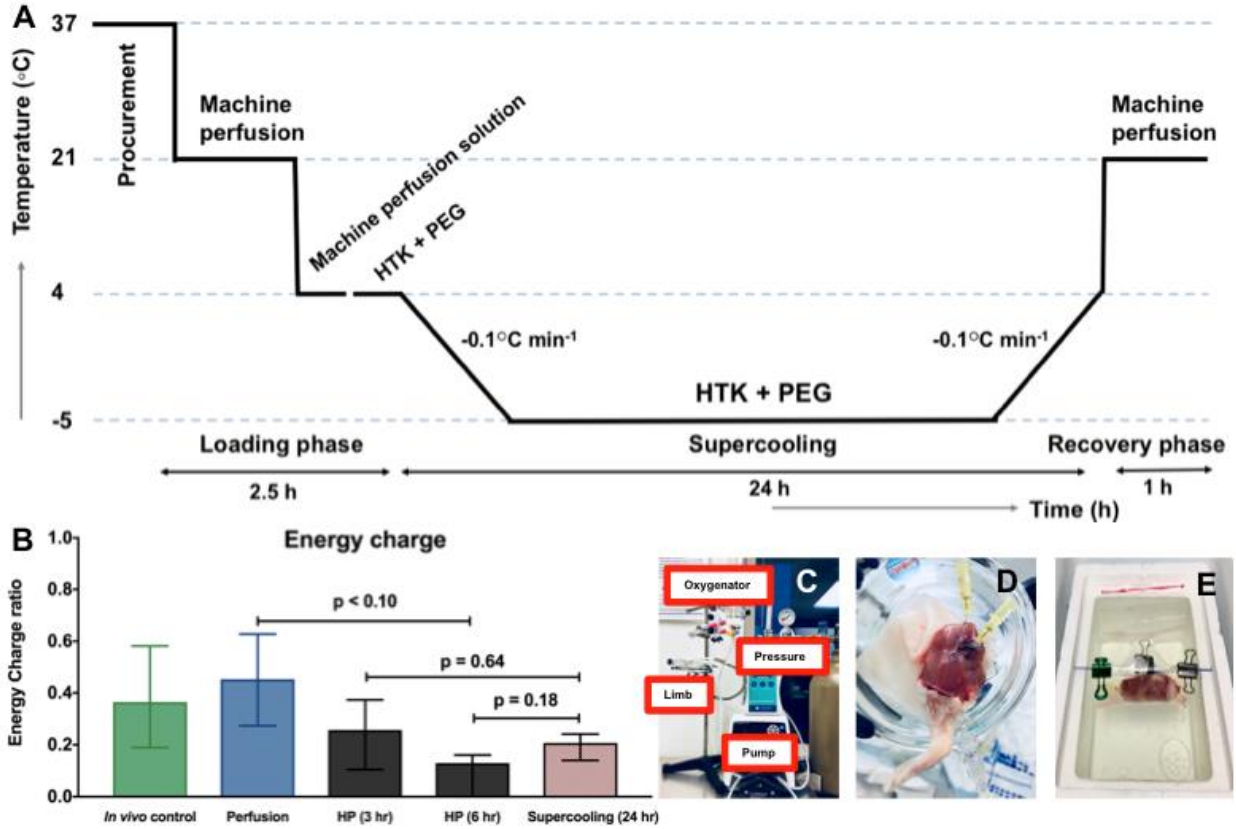

Figure 7: Successful supercooled preservation of rat hind limbs extends viable preservation to 24hrs. **A)** Our optimized protocol that includes a 2 hour perfusion loading phase. During this phase rat limbs are perfused with a solution based on PromoCell skeletal muscle media, 3-OMG, 3%BSA, 5%PEG, epidermal and fibroblast growth factors, heparin, insulin, antibiotics, L-glutamine, dexamethasone, hydrocortisone, prostaglandin. Afterwards, limbs are cooled to 8ºC with cold flush of the same solution (30 min). Then, the limbs are flushed with the supercooling solution based on HTK and PEG before storing them in a chiller and lowering the temperature to -5ºC. After supercooling, limbs are recovered using again machine perfusion with a solution similar to the loading phase but without 3-OMG and with an acellular oxygen carrier. **B)** Energy content was calculated based on mass spectrometry analysis (Energy charge = (ATP + 0.5ADP)/(ATP/ADP/AMP). We have previously demonstrated that energy charge is an indicator of graft dysfunction clinically and in rat liver transplant studies. As the energy charge displays, supercooling can extend the viable preservation time to 24hours. **C)** Our optimized perfusion system for pressure-controlled SNMP (21ºC) of rat hind limbs; **D)** Rat hind limb during machine perfusion. **E)** Hind limb during supercooling. All comparisons by ANOVA at $p<0.1$.

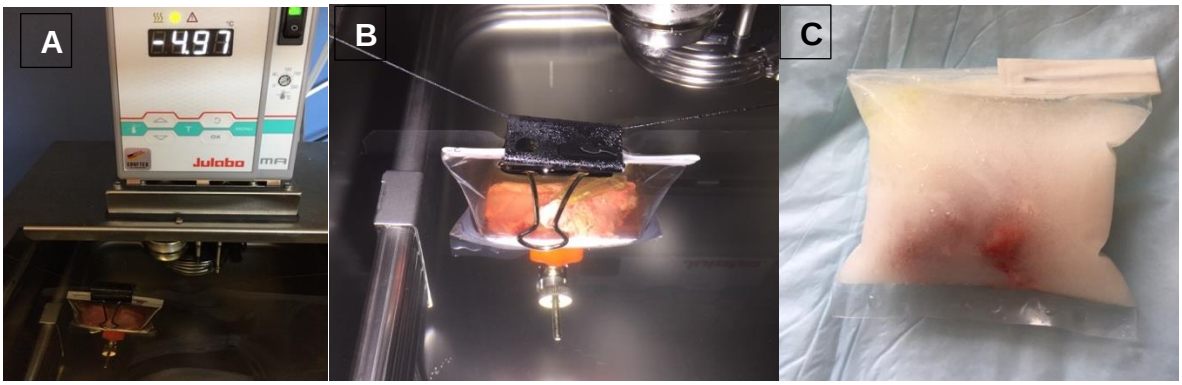

Figure 8: Supercooled preservation of a rat hindlimb. A& B) the limb stored in the chiller at -5°C. C) a frozen control limb for illustration purposes.

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

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Fellows, technicians and students have received microsurgery training from Dr. Cetrulo and his surgical team, including operating under magnification. Additional training by Drs. Uygun and Cetrulo included design of experiments, conduct of research, data collection and record keeping as well ethical and regulatory training on research. Staff also did and received mentorship on, and participated in writing of meeting abstracts, progress reports, manuscript drafts, as well as preparation and presentation of oral and poster talks at meetings.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

We presented the results of this research in three meetings relevant to the field. The first manuscript describing the results of perfusion protocol development was submitted and we are currently revising per reviewer suggestions; the main request was a larger number of transplants which are recently completed as shown in Fig 5 above. Two other papers are in preparation (one methods paper, one supercooling protocol original research paper). We also are working on an opinion

paper forecasting the potential number of VCA transplants in the US, if and when extended preservation and tolerance induction technologies are available. See *Products* below for detailed list.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

As noted in quarterly reports there were initial issues in the transplant model, and therefore our first goal is to complete the remaining perfusion transplants. The remainder of Year 3 will focus on testing the full supercooling protocol with transplants, and optimizing based on results.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The key accomplishment in year 2 is the development of a limb supercooling protocol, which we have shown to be successful based on tissue energy charge. To our knowledge this is a first in the field of VCA transplantation in terms of utilizing high subzero temperatures.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

As an interdisciplinary project, the results are expected to have impact on the fields of plastic surgery, transplantation, biopreservation, and medical systems engineering.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

We filed a new IP disclosure based on the results and the limb-specific protocol designed. On translational impact, results of this award also served as preliminary data for a new funded grant, which aims to scale up the technologies developed here to a porcine model in preparation for clinical trials.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

A combination of new research developments in this field, including results of this grant as well many other projects and efforts of other groups, is leading to a broad appreciation of the importance of preservation technology in transplantation. These together have already led to the formation of a new Community of Practice at American Society of Transplantation (AST), the Recovery and Preservation Community of Practice (RAP COP), currently chaired by the PI. As part of AST, the RAP COP provides feedback on organ allocation and transportation regulatory policies, and brings leadership in specific instances of bringing technology to clinical practice. Therefore, as part of a larger consortium of projects, and together with other groups including organ procurement organizations, advocacy organizations and professional societies, the work is affecting regulatory decision making, clinical and business practices, which in turn is expected to increase the utilization of life altering transplantation technologies to improve social and economic conditions in the US and beyond.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

No significant changes. As detailed in quarterly reports, we observed excessive edema in early experiments, which required a retuning of the perfusion media and protocols. We are currently finalizing testing of the new protocol with transplants, which focuses on using hyperosmolal media to minimize edema.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We experienced two distinct issues with the transplant models. First, self-mutilation of the transplanted grafts was a confounding factor for evaluation. Based on both clinical observations and histology results, we are now considering self-mutilation as a clinical marker of nonviability; developing a more robust scoring criteria is still in process.

Second, our results indicate that the rat hindlimbs, in particular the blood vessels, are especially sensitive to both shear damage and ischemia-reperfusion injury. This leads to a higher technical error rate than initially expected based on prior rat liver and porcine and primate VCA tissues, which led to some minor variations and more media iterations than initially planned to resolve these issues. For instance we performed an intermediate 3 hour perfusion group as this was the time point where significant edema was starting to reveal. We currently have a protocol that we consider good-enough to perform planned studies. We will also test if the edema issues continue in the porcine studies in a parallel project; our experience as well as discussions with colleagues in the field indicate the large vessels in porcine grafts are much more robust, and we expect this issue will be less critical as we scale up.

Consequently, we are somewhat behind in the transplant testing of the protocols. To accommodate for that we will prioritize the key control and experiment groups for testing, so that the studies at the end of year 3 will produce a viable protocol with success evaluated with sufficient statistical power.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

The current media costs are significantly higher than the initial estimated amounts, and the issues with transplants also led to increased personnel-time. Consequently the grant is currently overspent. We are finalizing testing of the new perfusion protocol which we hope will reduce the cost of media supplements. While we do not expect this increased cost will affect the current project significantly, the increased perfusion costs may impact a parallel project which scales up the protocol to porcine grafts.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

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**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. O Mamane, LY chin, AG Lellouch, M Randolph, K Uygun, C Cetrulo, B Parekkadan. Tracking of the Engineered Fibroblasts in Vascularized Composite Allograft: A proof-of-concept study. Presented at the Biomedical Engineering Society (BMES) Annual meeting, Philadelphia PA, Oct 16-19, 2019. DoD support acknowledged.
2. LC Burlage, AG Lellouch, GG Saviane, MA Randolph, L Lantieri, RJ Porte, SN Tessier, K Uygun, CL Cetrulo. First Vascularized Composite Allotransplantations in Rats after 6 Hours of Ex Vivo subnormothermic Machine Perfusion Using a Hemoglobin Oxygen Carrier: A Proof of Concept Study. Journal of Burn Care & Research, 40 (S1), 133-134, 2019. DoD support acknowledged.
3. LC Burlage, AG Lellouch, O Mamane, MA Randolph, L Lantieri, RJ Porte, SN Tessier, K Uygun, CL Cetrulo. First Successful 24 Hours Preservation in Vascularized Composite Allograft Using a Subzero Non-Freezing Protocol. Presented at the American Society for Reconstructive Microsurgery Annual meeting, Palm Desert CA, Feb 1-5 2019. DoD support acknowledged.
4. RJ de Vries et al. Supercooling extends preservation time of human livers. Nature Biotechnology, 37, 1131-1136, 2019. DoD support acknowledged.
5. Burlage et al. Optimization of Subnormothermic Machine Perfusion for Ex Vivo Preservation of Rodent Vascularized Composite Allografts. Transplant International, in revision 2019. DoD support acknowledged.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation);*

status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report.

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

Nothing to Report.

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

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

A novel protocol for limb supercoooling was developed.

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

An IP disclosure was filed to MGH based on the new limb-specific protocol developed.

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

- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: *Korkut Uygun*
Project Role: *PI*
Nearest person month worked: *1*
Contribution to Project: *Co-led the project*

Name: *Curt Cetrulo*
Project Role: *Co-PI*
Nearest person month worked: *1*
Contribution to Project: *Co-led the project*

Name: *Mark Randolph*
Project Role: *Investigator*
Nearest person month worked: *1*

Contribution to Project: *Assisted in IACUC protocol development, experimental design, planning for animal studies, training of personnel in small animal surgery, anesthesia, limb transplantation.*

Name: *Alexandre Lellouch*
Project Role: *Fellow*
Nearest person month worked: *4*
Contribution to Project: *Led the graft recoveries and transplantations, experimental design with Co-PIs*

Name: *Shannon Tessier*
Project Role: *Fellow/Investigator*
Nearest person month worked: *1*
Contribution to Project: *Led development of the new perfusion cocktail*

Name: *Peony Banik*
Project Role: *Research Technician*
Nearest person month worked: *7*
Contribution to Project: *Assisted in perfusion studies and data collection*

Name: *Paria Mahboub*
Project Role: *Research Specialist*
Nearest person month worked: *2.5*
Contribution to Project: *Assisted in perfusion studies and assessment*

Name: *Stephanie Cronin*
Project Role: *Research Technician*
Nearest person month worked: *4*
Contribution to Project: *Performed biochemical assays*

Name: *Casie Pendexter*
Project Role: *Research Technologist*
Nearest person month worked: *4*
Contribution to Project: *Performed and assisted in graft recoveries, perfusion and transplants*

Name: *Olivia Mamane*
Project Role: *Student/Temp*
Nearest person month worked: *6*
Contribution to Project: *Trained as perfusionist, writing a review/opinion paper on VCA transplant under supervision of Drs. Cetrulo and Uygun*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

See attached Other support documentation. No effects on the effort in this project.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

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

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

See attached quad chart.

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.*

No additional documents to report.