AWARD NUMBER: W81XWH-19-1-0744

TITLE: A Novel Application of Normothermic Machine Perfusion for Face Recovery to Reduce Intragraft Inflammation and Optimize Organ Viability

PRINCIPAL INVESTIGATORS: Gerald Brandacher, M.D.

CONTRACTING ORGANIZATION: Johns Hopkins University School of Medicine

REPORT DATE: October 2021

TYPE OF REPORT: Annual Technical Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2021	Annual Technical Report	9/1/2020 – 8/31/2021

4. TITLE AND SUBTITLE

A Novel Application of Normothermic Machine Perfusion for Face Recovery to Reduce Intragraft Inflammation and Optimize Organ Viability

5a. CONTRACT NUMBER
W81XWH-19-1-0744

5b. GRANT NUMBER
RT180059

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

John Brassil E-mail: jbrassil@functionalcirculation.com
Gerald Brandacher M.D. E-Mail: gbranda2@jhmi.edu

E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Functional Circulation LLC
326 Hatchery Lane
Lake Mills, WI 53551

Johns Hopkins University
733 N. Broadway, Ste. 117
Baltimore, MD 21205-1832

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development 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

14. ABSTRACT:

Warriors who suffer from combat-related injuries. Face transplantation is currently the only treatment option to fully restore devastating craniofacial injuries with functional and anatomical equivalents by replacing "like-with-like" tissue. Recent advances in microsurgical techniques and immunosuppressive protocols have enabled wider application of face transplantation with highly encouraging results. However, the current gold standard in tissue preservation – static cold storage on ice – is insufficient to preserve facial allografts for more than a few hours. Advancements in the field of VCA regarding matching and allocation, desensitization, and potential tolerance induction are all within reasonable reach to achieve; these are, however, constrained by limited preservation time. Thus, this project applies normothermic machine perfusion (NMP), as practiced clinically in vital organ transplant, to the specific and unique requirements of the face to increase tissue viability and to reduce both ischemia reperfusion injury and inflammatory potential.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	23	USAMRMC

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. Introduction

Vascularized composite allotransplantation (VCA) holds much promise to improve the quality of life for our Wounded Warriors who suffer from combat-related injuries. Face transplantation is currently the only treatment option to fully restore devastating craniofacial injuries with functional and anatomical equivalents by replacing *"like-with-like"* tissue. Recent advances in microsurgical techniques and immunosuppressive protocols have enabled wider application of face transplantation with highly encouraging results. However, the current gold standard in tissue preservation – static cold storage on ice – is insufficient to preserve facial allografts for more than a few hours. Advancements in the field of VCA regarding matching and allocation, desensitization, and potential tolerance induction are all within reasonable reach to achieve; these are, however, constrained by limited preservation time. Thus, this project applies normothermic machine perfusion (NMP), as practiced clinically in vital organ transplant, to the specific and unique requirements of the face to increase tissue viability and to reduce both ischemia reperfusion injury and inflammatory potential.

2. Keywords

VCA, normothermic machine perfusion, sub-normothermic machine perfusion, face transplantation, preservation

3. Accomplishments

- **What were the major goals of the project?**

Site 1:

The Site 1 project goals are to adapt, develop and support three normothermic perfusion machines for preservation of faces and hemifaces up to 24 hours. Two of the machines are benchtop systems (Fig.1) that enable Site 2 porcine hemiface perfusion and transplant studies comparing normothermic perfusion of 12 and 24 hours to multithermic perfusion and to the gold standard, cold static storage. A portable machine (Fig.2) to perfuse faces donated for research, will be transported while perfusing to the lab for evaluation. This portable system is intended for preliminary validation in a clinically-realistic application. Throughout the project, Site 1 furthermore supports the experimental program by providing perfusate and perfusion kits plus making improvements to the systems based on learned lessons. As a result of this project, investigators aim to improve the viability of preserved faces, including reduced potential for inflammation that will result in better matching over greater distance, reduced requirement for immunosuppression, and improved graft function.

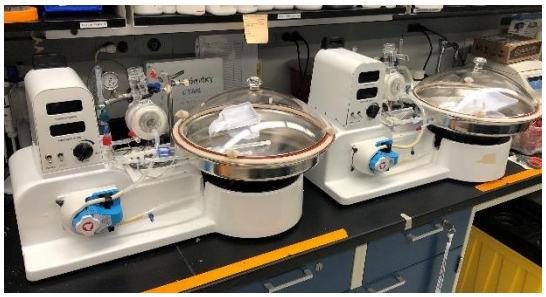


Figure 1. Benchtop Perfusion Systems

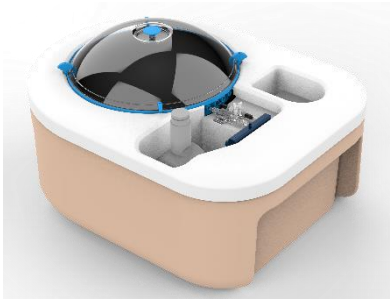


Figure 2: Portable System Rendering

Site 2

Activities were focused on performing biochemical and histological testing on grafts and control tissues at specified timepoints (hours 1, 2, 6, 8, 20, 24) during perfusion and at the experimental end. In addition to these results were reviewed to modify/revise perfusion system as necessary to optimize tissue preservation. Literature review and internal preparations were being made to optimize the perfusion and surgical procedures in collaboration with Site 1.

Subsequent to this, activities were focused on finalizing the surgical model to allow us to proceed to Major Task 2, Subtask 2 Perform orthotopic facial transplantation using a swine leukocyte antigen (SLA) defined minipig model with major histocompatibility match with tacrolimus maintenance using grafts stored for 12 hours in static cold storage (Group I; control group) and Subtask 4: Perform orthotopic facial transplantation using a swine leukocyte antigen (SLA) defined minipig model with major histocompatibility match with tacrolimus maintenance using grafts perfused for 24

hours of NMP (Group III; experimental group). During execution of this step, setbacks in explanted transplant viability were noted, initiating a review of protocols.

Following these setbacks, Site 2 activities were focused on Major Task 1, Subtask 4: Perform hemiface graft explants for testing on new perfusion device; Major Task 1, Subtask 5: Perform biochemical and histological testing on grafts and control tissues at specified timepoints (hours 1, 2, 6, 8, 20, 24) during perfusion and at the experimental end [H&E, IHC, glucose, lactate, pH]; and Subtask 6: Review results from Subtask 3-5 and revise perfusion system as necessary to optimize tissue preservation. In anticipation of renewed progression to Major Task 2 in the coming reporting period, literature review and internal preparation are currently being conducted.

Specific Aim 1: Design and produce a perfusion system that applies sustained normothermic, pulsatile perfusion, active disinfection, and ergonomic support to enable 24-hour, prolonged preservation of faces.	**Timeline**	**Site 1** (Initiating PI)	**Site 2** (Partnering PI)	**Progress**
Major Task 1: Development of benchtop normothermic perfusion systems adapted for 24-hour perfusion of the face.	Months	Functional Circulation (Brassil)	Johns Hopkins (Dr. Brandacher)	%
Subtask 1: Design and develop two normothermic perfusion systems for concurrent preservation of porcine hemifaces including component design for temperature modulation, pressure control, oxygenation, automation, and combination disposable/reusable perfusion circuit with passage of Assembly and Factory Acceptance Test	1-3	X		100
Milestone #1: Perfusion systems ready for Major Task 1, Subtask 3				
Subtask 2: Submit documents for Institutional Animal Care and Use Committee (IACUC) and DoD Animal Care and Use Review Office (ACURO) approvals	1-3		X	100
Milestone #2: IACUC and ACURO approval obtained				
Subtask 3: Provide and support the perfusion systems for initial testing of the perfusion system on porcine hemifaces	4-6	X		100
Subtask 4: Perform hemiface graft explants for testing on new perfusion device [4-6 hemifacial grafts]	4-6	x	X	100
Subtask 5: Perform biochemical and histological testing on grafts and control tissues at specified timepoints (hours 1, 2, 6, 8, 20, 24) during perfusion and at the experimental end [H&E, IHC, glucose, lactate, pH]	4-6		X	100
Subtask 6: Review results from Subtask 3-5 and revise perfusion system as necessary to optimize tissue preservation	7-8	X	X	100
Milestone #3: Perfusion systems ready for Major Task 2				
Specific Aim 2: Perform orthotopic transplantation of SLA-matched and mismatched porcine hemifaces.				
Major Task 2: Explant, preserve, transplant, and manage and observe recovery of swine hemiface recipients (n = 25 recipient swine, n = 13 donor swine)				
Subtask 1: Provide and support two perfusion systems to perform hemiface preservation	8-24	X		40
Subtask 2: Perform orthotopic facial transplantation using a swine leukocyte antigen (SLA) defined minipig model with major histocompatibility match with tacrolimus maintenance using grafts stored for 12 hours in static cold storage (Group I; control group) [5 recipient pigs total]	8-11	X	X	40
Subtask 3: Perform orthotopic facial transplantation using a swine leukocyte antigen (SLA) defined minipig model with major histocompatibility match with tacrolimus maintenance using grafts perfused for 12 hours of NMP (Group II; control group) [5 recipient pigs total]	11-14	X	X	
Subtask 4: Perform orthotopic facial transplantation using a swine leukocyte antigen (SLA) defined minipig model with major histocompatibility match with tacrolimus maintenance using grafts perfused for 24 hours of NMP (Group III; experimental group) [5 recipient pigs total]	15-18	X	X	40
Subtask 5: Perform orthotopic facial transplantation using a swine leukocyte antigen (SLA) defined minipig model with major histocompatibility match with tacrolimus maintenance using grafts	19-22	X	X	40

perfused for 24 hours sequentially comprising of NMP, hypothermic machine perfusion, and NMP (Group IV; experimental group) [5 recipient pigs total]				
Subtask 6: Perform orthotopic facial transplantation using a swine leukocyte antigen (SLA) defined minipig model across a full major histocompatibility mismatch with induction therapy and tacrolimus maintenance using grafts perfused for 24 hours sequentially comprising of NMP, hypothermic machine perfusion, and NMP (Group V; experimental group) [5 recipient pigs total]	22-24	X	X	
Subtask 7: Perform protocol skin biopsies from healthy pig skin, experimental, and control groups and evaluate histopathological changes (H&E, IHC).	8-24		X	20
Subtask 8: Perform biochemical monitoring during graft perfusion (Glucose, lactate, pH)	8-24		X	20
Subtask 9: Perform immune cell phenotyping, metabolic monitoring (CBC, LFT, Cr, BUN, glucose), and immune monitoring assays (Luminex, CFSE MLR) on Group V animals	8-24		X	
Subtask 10: Analyze and compare the data from Groups I-V to assess for optimal perfusion system and settings	24	X	X	
Milestone #3: Experimental data collected and analyzed				
Specific Aim 3: Apply the developed approaches of 24-hours normothermic preservation with viability assessment to human faces donated for research.				
Major Task 3: Explant, preserve, and analyze human faces donated for research (n = 2-3)				
Subtask 1: Apply for HRPO and IRB approval	15-24		X	
Milestone #4: HRPO approval received				
Subtask 2: Contract with local OPO to obtain faces donated for research	15-24		X	
Subtask 3: Design, assemble, and qualify portable perfusion system for faces	19-24	X		30
Milestone #5: Approvals, agreements, and systems in place for feasibility study of portable perfusion preservation of human faces				
Subtask 4: Recovery, preservation, and analysis of human faces normothermically preserved on the portable system	25-33	X	X	
Subtask 5: Compile Design Outputs for FDA Design History File	33-36	X		70
Milestone #6: Feasibility study with human faces complete				
Subtask 6: Write and submit results for publication	33-36	X	X	

- **What was accomplished under these goals?**

Site 1:

Major Activities

In the second project year that we are just concluding, the project focused on perfusing porcine hemifaces followed by transplantation. For Site 1, the system we provided for initial feasibility studies was adapted for its implementation role in this transplant phase. We engaged with electrical engineers to design the main CPU board and the battery module. We engaged with an industrial designer to develop concepts for the portable system. We engaged with tooling engineers for design review of the disposable set and to evaluate molding vendors. And we engaged with a regulatory consultant to work with us on the risk analysis. On the basis of these engagements, we developed our perfusion machines into production versions that we assembled and verified.

We received feedback from Site 2 for design improvements based on their observations in the experimental program. As a result, we improved the system's cannulation, organ support, and datalogging capabilities.

On the bench at Site 1, we studied perfusate chemistry stability in recirculating dialysis, and microbe inhibition by UV irradiation of perfusate in laminar flow conditions. These studies showed promise and became the subject of a pending US patent.

Specific Objectives

Our major objective for this recent second year of the three year project was to support existing hemiface perfusion machines for porcine allotransplantation studies at Site 2. This objective included designing and producing a single-use disposable organ holder and tubeset (Fig.1) to streamline the experimental process. Within this major objective, we focused on hardening the systems for improved reliability, incorporating new datalogging features, cannula, hemiface support surface, and software improvements.

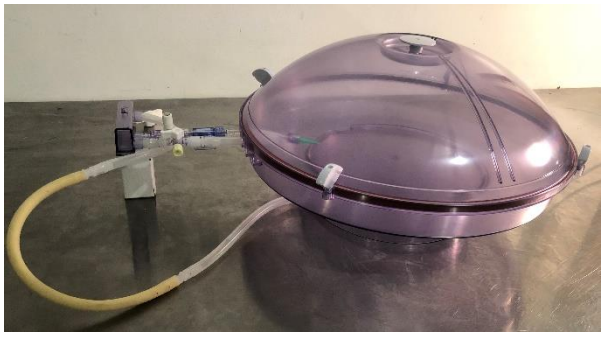


Figure 1. Single Use Tubeset

A second objective was to develop a portable system for normothermically perfusing human faces donated for research. This development is underway with key subsystems complete including disposable tubeset, battery module, printed circuit board and wiring. Work on this objective remains in process: frame, enclosure, and closed loop pressure control are yet to be completed.

A third objective was to compile Design Outputs for the FDA Design History File. This objective is on track as the key element, the Risk Analysis is complete. Further compilation remains to be done as the design materializes in this coming year.

Significant Results or Key Outcomes

Two benchtop perfusion systems were delivered and maintained at Site 2 for normothermic preservation of porcine hemifaces per the aims of the project. A disposable organ holder and integrated tubeset, sized for the faces and hemifaces of this project (360 mm diameter) was designed, quoted, injection molds made, molded, and assembled. This disposable set is ready for final validation and transfer to Site 2 for implementation in the experimental program. It incorporates biocompatible, medically-approved materials that can be sterilized by ethylene oxide or gamma methods.

Continuous improvement was implemented, wherein feedback from Site 2 was incorporated into the system as refinements including improved datalogging, cannulas, face support surface, perfusate swap. These improvements are outlined below.

Other Accomplishments

A method of recirculating dialysis was developed and tested (Fig.2). Dialysate recirculation enables portability and provides a practical means for removing unwanted metabolic products such as lactate from the perfusate. We show removal of lactate (Fig.3) while maintaining stable perfusate chemistry by a recirculating process of dialysis with ion exchange. To date this process has been demonstrated on the bench. It is a subject of a pending patent.

Figure 2. Dialysis

A method of preventing microbial expansion within the perfusate by ultraviolet irradiation (Fig.4) during perfusion also has been demonstrated on the bench. The effect on the perfusate erythrocytes is still in evaluation, as poikilocytosis (Fig.5) was observed (although this is a common effect in porcine blood). Ultraviolet control software has been updated to prevent emissions when the perfusate is stopped or slow-flowing to prevent its overheating. This module is also a subject of a pending patent. Notice of patents pending has been made through the iEdison system as required.

Improvements in datalogging have been made in phases. A cloud based datalogging capability was set up initially, whereby data was sent via Wi-Fi to a hotspot, from which it was sent to a cloud server and then retrieved and archived by a computer at Site 1. From there, the archive is made available on Dropbox, and retrievable by authorized team members. Due to intermittencies with Wi-Fi, the cloud interface is being upgraded to a cellular connection. This will be a useful mode of connection for the portable system. Additionally, we have placed a computer inside the benchtop system for high volume data gathering. By connecting a flash drive to its USB port, files may be directly and immediately retrieved at the lab. This high capacity data acquisition capability will be beneficial in future machine learning applications.

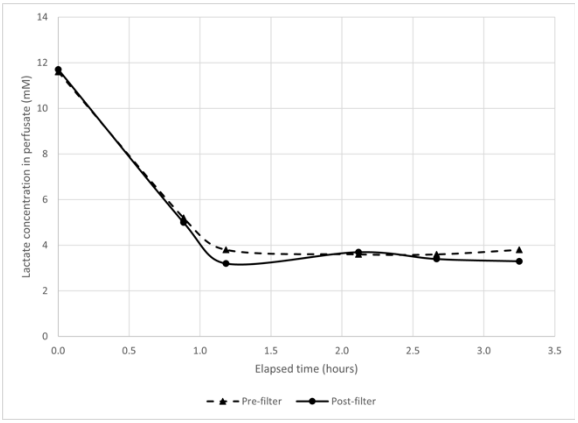


Figure 3. Lactate Reduction in Dialysis

The wiring and electronics of the benchtop systems are being replaced by factory-manufactured wiring harness and printed circuit board. We are updating one of the benchtop systems now, for imminent shipment back to Site 2 following verification. The other system will be revised subsequently.

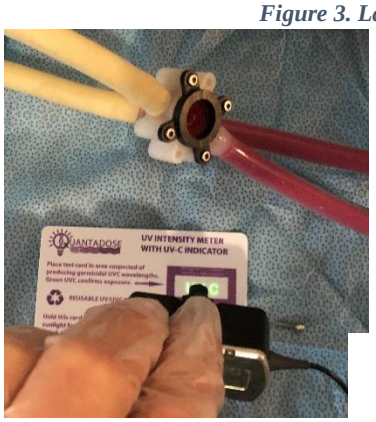


Figure 4. Disinfector and Cuvette

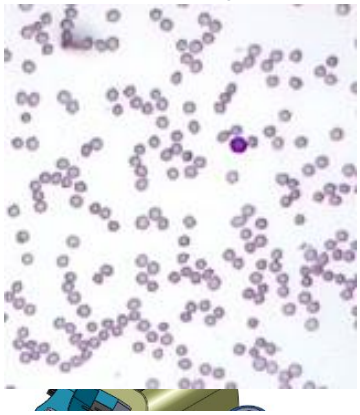


Figure 5. Porcine RBCs After UV

A module for selectively performing aseptic perfusate swap (Fig.6), or controlled infusion, or perfusate dialysis has been designed and is in fabrication. This module would be placed next to the benchtop system and connected to the tubeset. It can be run in forward or reverse mode at controlled speed and pressure to infuse or remove material from the perfusion circuit. Further it can be set to recirculate for dialysis. This module is on order and will be assembled and ready in the upcoming quarter.

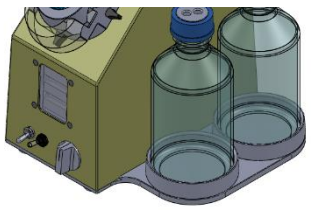


Figure 6. Perfusate Swap Module

Cannula refinements (Fig.7) were requested by Site 2, to reduce the length of a standard venous cannula, without leaving a sharp or serrated edge that otherwise occurs by cutting with a scalpel or scissors. We developed a method of making a square cut cannula tip followed by a solvent treatment to round the cut edge. We provided a range of cannula sizes (15 to 22 ga.) modified for short blunt tip,

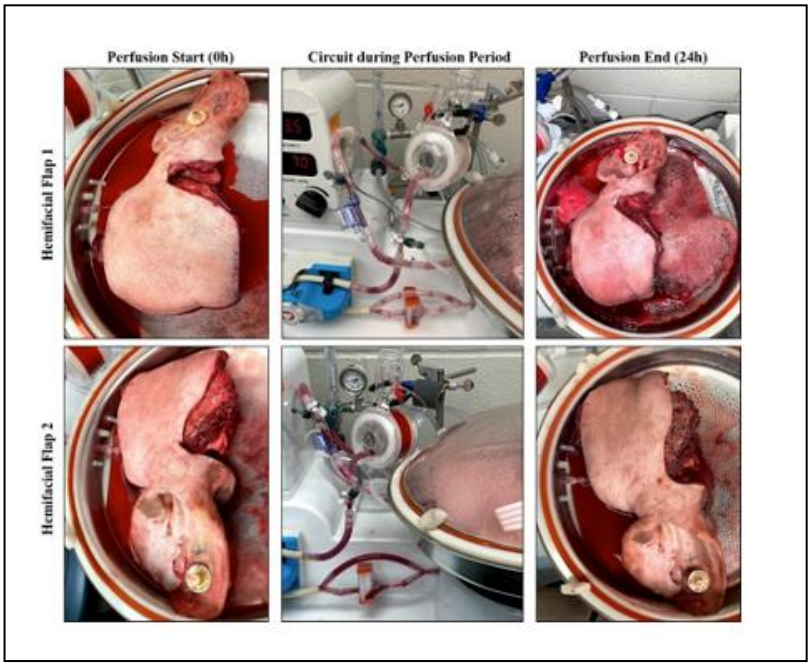
A face support surface was proposed by Site 2 to enhance venous drainage of the porcine hemiface by elevating one end. We fabricated two versions ready to share with the Site 2 team for evaluation and refinement.

Figure 7. Cannula refinement

Site 2

At the end of the previous annual reporting period, Site 2 performed a second hemifacial graft harvest operation with the help of a preoperative cone beam CT-angiography to provide guidance on graft anatomy. Two hemifacial flaps were explanted, flushed, and weighed before being placed in the enlarged reservoir for 24-hour normothermic perfusion with a modified autologous whole blood based perfusate. Flap 1 was placed on a continuous perfusion circuit and Flap 2 was placed on a pulsatile perfusion circuit (Figure 8). Flap 1 on the continuous perfusion pump had one technical failure at hour 4 of perfusion which caused a loss of perfusate volume as well as air entry into the circuit.

Figure 8: Hemifacial grafts pre- and post- perfusion. Top Row (left to right): Hemifacial flap 1 at beginning of perfusion, air entry into circuit, graft with evidence of air embolism and thrombosis. Bottom row (left to right): Hemifacial flap 2 at beginning of perfusion, appropriate appearance of circuit during perfusion, and 24 hour normothermically perfused graft.



Building on this, Site 2 focus during this annual reporting period began with the completion of Major Task 1 surgeries and finalizing the hemifacial transplant operation to allow work on Major Task 2. On February 16, 2021, the final set of two hemifacial grafts were explanted from a single pig as a terminal procedure for a 24-hours perfusion run on bioreactors provided by Site 1. On this day, refinement of the graft harvest procedure was the main goal. Pre-operative CT angiography was again performed to assist in flap planning. Critical steps and landmarks are demonstrated in Figure 6 below.

As shown in Figure 9, the skin was marked starting at the anterior ear, down and around inferior border of zygoma to the inferior border of the mandible (approximately 5cm from inferior border of the mandible and 5cm from posterior border of the mandible) and then up and around the ear (Figure 9A). The key landmark that must be identified to locate the flap vasculature is the tip of the styloid process (Figure 9B), which can be found by coming around the ear (Figure 9C), along the skull and following the bone deep to a large bony prominence where there are multiple muscular insertions (Figure 9D). This is the tip of the styloid process. The superficial temporal artery and other flap vessels run superficial to the styloid process and can be traced caudally (Figure 9E) between the paraspinous muscles and the airway strap musculature. In this region of the lateral neck, the sternocleidomastoid muscle (SCM) can be identified. There, a superficial vein was noted to run along the outside of the SCM. This was the external jugular vein (EJV) which is the major venous outflow for the hemifacial flap. The SCM was transected, and the vein was retracted superiorly to allow the identification of the lingual artery, which is also transected. Next, the inferior border of the mandible was identified and cut (Figure 9F), with care taken to stay just below the condyle to avoid injury to the flap vessels just anterior to the styloid and posterior to the posterior border of the mandible. This allowed for dissection deep along the bone of the muscle containing these vessels (Figure 9G) until the pterygoids were reached and cut, allowing the pedicled flap to be completely mobilized and ready for harvest (Figure 9H).

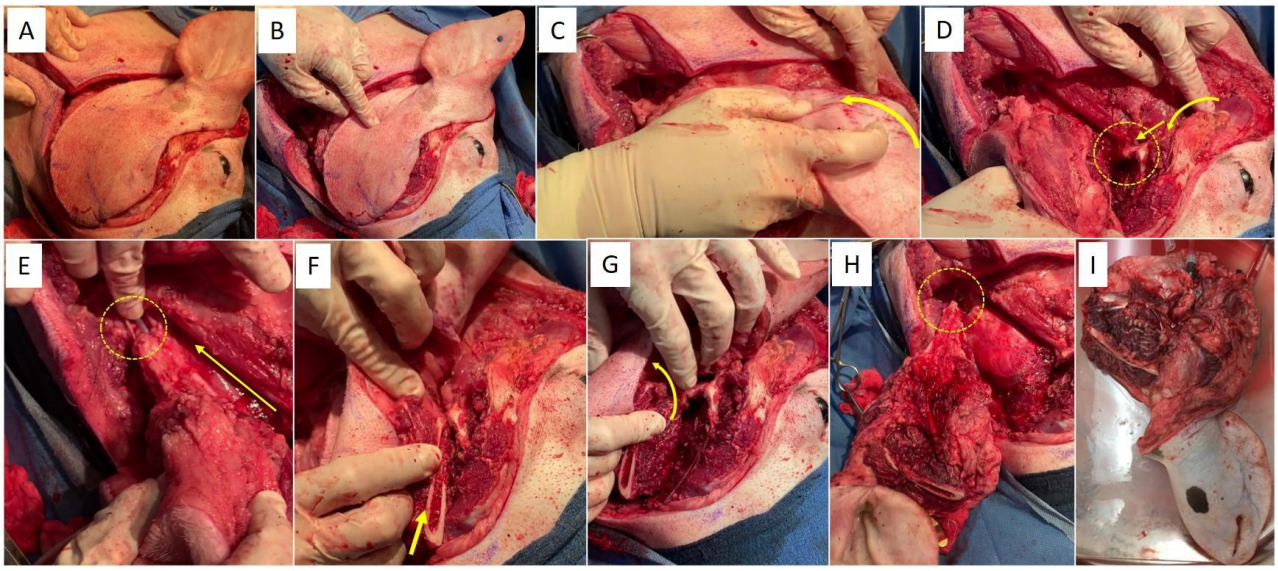
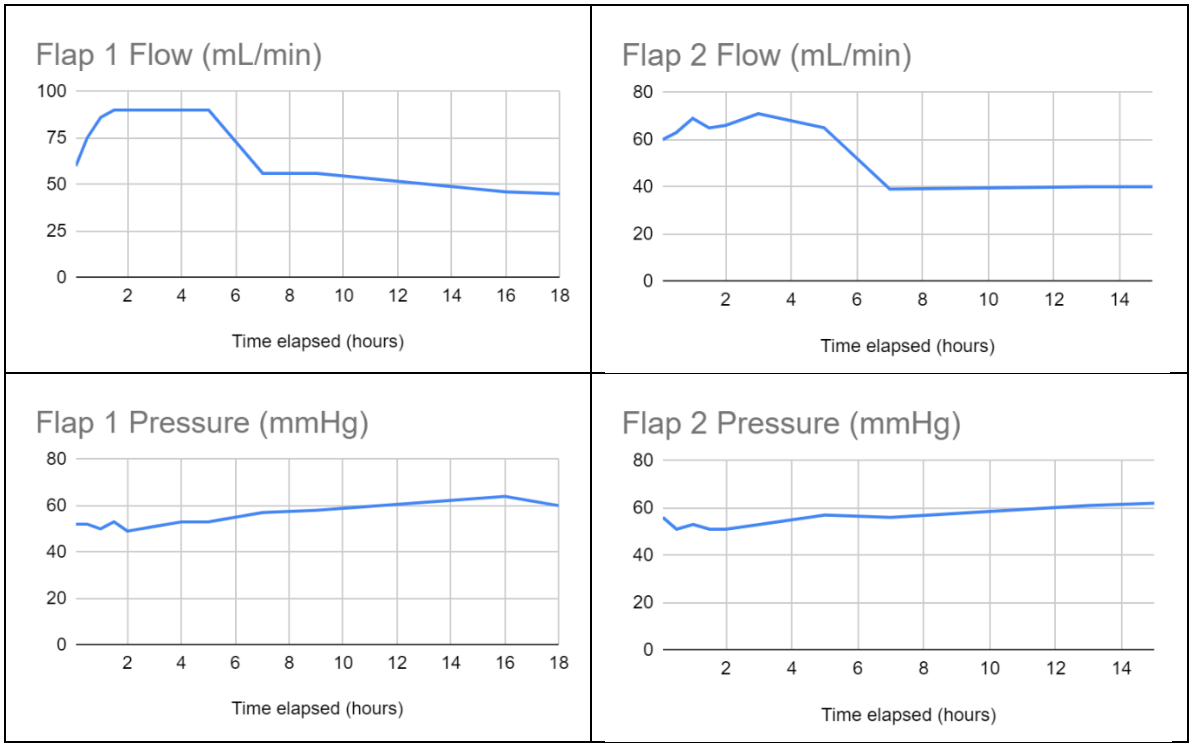


Figure 9: Hemifacial Graft Harvest Procedure. A) Skin incised over initial skin marking. B) Approximate location of styloid process. C)-D) Path to styloid process from anterior ear (yellow arrows). Styloid process

Both Flaps were perfused for 24 hours normothermically. A blood based perfusate was again used for perfusion. Representative images of the flaps during perfusion are shown in Figure 8. Greater than 30% edema was noted in both flaps after 24 hours.

The reason for this is suspected to be a 3.5% albumin concentration of the perfusate (compared to closer to 6% in the previous perfusion experiment days). The flap positioning (with muscle-side facing up) may also have contributed to a slightly different drainage pattern with this perfusion run compared to the previous runs. The greater edema most likely contributed to the increased flap vascular resistance shown in Figure 10.

Flap hemodynamics were closely monitored during this perfusion run using the cloud-based data logging capability for the perfusion systems delivered by Site 1. Vascular resistance data for these flaps is shown in Figure 10.



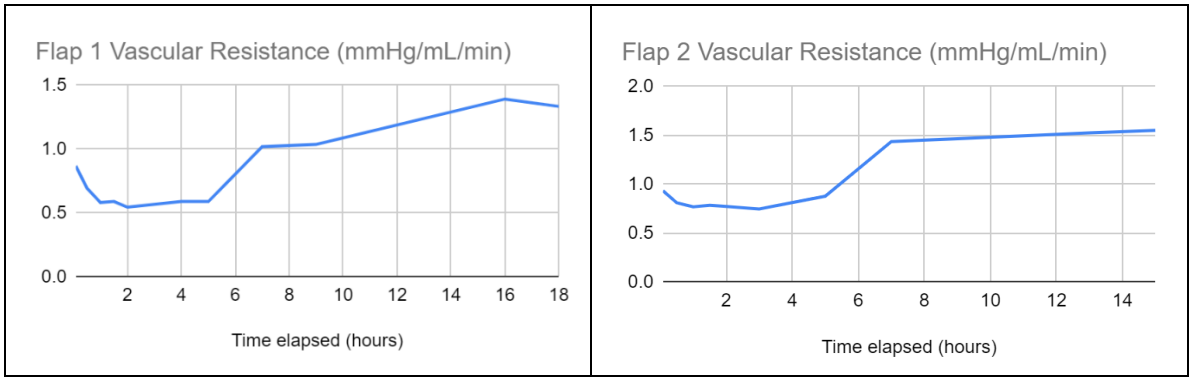


Figure 10: Flap Hemodynamics Over 24hr NMP – 2.16.21.

Under Major Task 1 Subtask 5 perfusion chemistries were monitored for both flaps over the 24-hour perfusion period (Figure 11). Based on observed chemistries, perfusate was adjusted to more closely resemble physiologic levels with bicarbonate, calcium gluconate and dextrose. This was based on literature suggesting potential benefit to replenishing nutrients that are depleted over the course of the prolonged perfusion period.

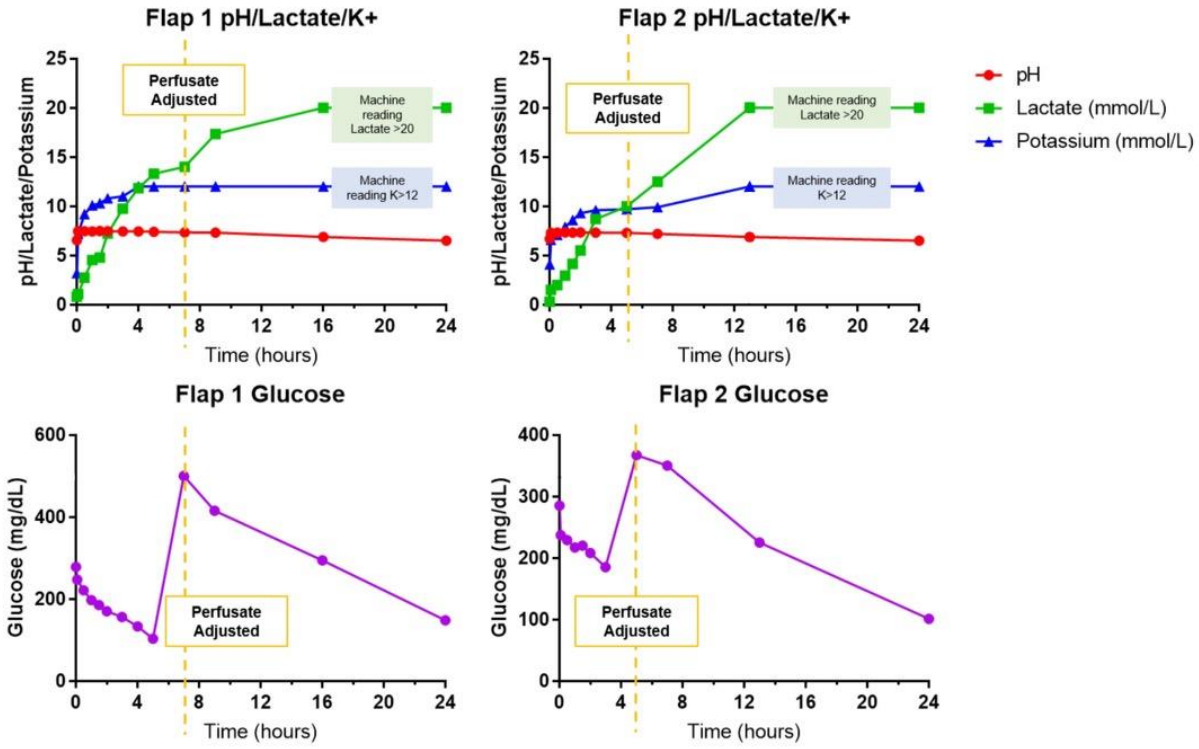


Figure 11: Flap Chemistries Over 24hr NMP – 2.16.2

In this case, the acid base status and lactate production patterns after the adjustment did not suggest any clear benefit to this. Site 1's dialysis feature is anticipated to resolve the issue of maintaining physiologic acid-base and electrolyte homeostasis through the extended *ex-vivo* perfusion period.

With the completion of Major Task 1 swine surgeries, Site 2 was able to move forward to Major Task 2 (Explant, preserve, transplant, and manage and observe recovery of swine hemiface recipients). On March 2, 2021, Site 1 harvested two hemifacial flaps from donor pig 25763 (SLA HH). The recipient swine 25761 and 25762 were both haploidentical littermates of the donor pig 25763 (SLA HH)

The donor dissection was again based on cone beam CT angiography. For this procedure, the plastic surgery team met with the diagnostic radiologist ahead of the surgery to obtain more detailed information on the 3-dimensional course of the flap vessels. Representative images mapping the facial vasculature resulting from this meeting are

shown in Figure 12. Images in Figures 10-A and B represent 2-dimensional views from the cone beam CT. Figure 10-C and D are taken from a 3-dimensional, 360° map of vessel trajectory relative to pig 25763's bony anatomy.

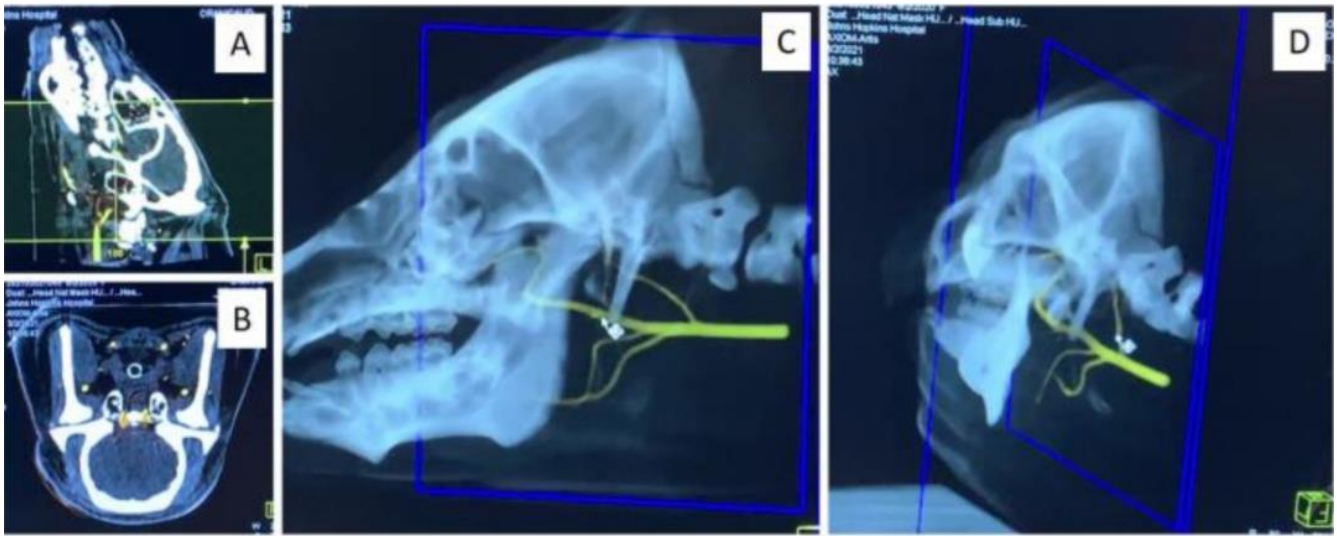


Figure 12: Swine 25763 Pre-Operative Imaging 3.2.21

The dissection on March 2 proceeded according to the harvest procedure described in Figure 9 above. One hemifacial flap was flushed in the operating room and then attached to the bioreactor for normothermic machine perfusion per Subtask 3 (Perform orthotopic facial transplantation using a swine leukocyte antigen (SLA) defined minipig model with major histocompatibility match with tacrolimus maintenance using grafts perfused for 12 hours of NMP). Due to the logistics of our large animal OR facility, the perfusion period was closer to 20 hours. Figure 13 shows the graft over the course of the perfusion period.

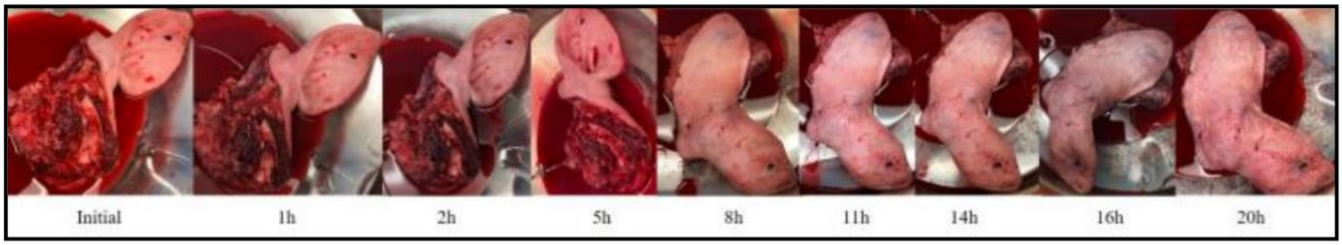


Figure 13: Hemifacial Flap NMP Clinical Monitoring 3.2.21.

In the first 1.5 hours of perfusion the graft did appear slightly congested although no hemodynamic signs of venous outflow obstruction were apparent. As a result, the vein was evaluated by the plastic surgery team. With some manipulation of the vein, the coloration of the graft did appear to improve slightly. At approximately 3.5 hours into perfusion, there was a sudden increase in the pressure and resistance and decrease in the flow. Hemodynamic data for this graft is shown in Figure 14. There was no manipulation immediately preceding this and immediate visual inspection of the graft did not reveal any evidence of outflow obstruction.

At this time, the graft was repositioned with muscle facing down and the tip of the venous outflow was trimmed to ensure no adventitial or other connective tissue could be obstructing the outflow lumen. The vein was gently flushed with heparinized saline as well. Hemodynamic parameters after this intervention reflected decreasing pressure and resistance and increased flow. However, this did not normalize to the levels seen prior to the resistance spike. Chemistries were also monitored and were unremarkable (data not shown).

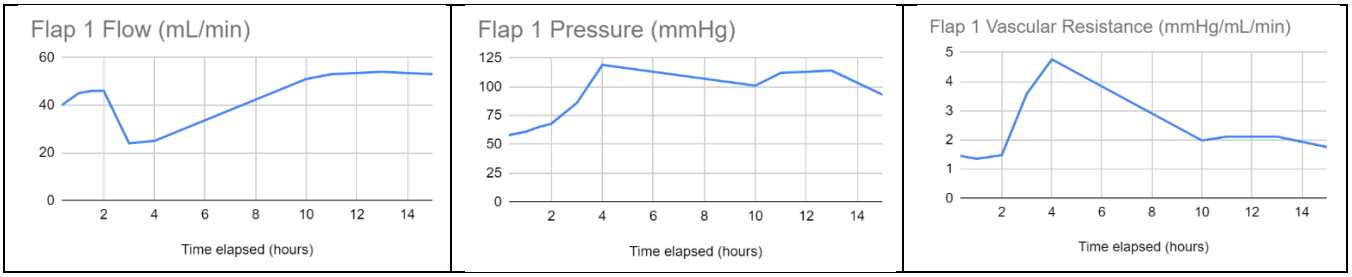


Figure 14: Hemifacial Flap NMP Hemodynamics 3.2.21. *Data gap issue being addressed by Site 1.

Overall flap appearance as well as hemodynamic parameters remained stable until about 14 hours of perfusion, when the flap skin appeared dusky and congested. Venous outflow was again evaluated at that time and outflow, albeit slow, was present. These skin changes persisted until the end of perfusion at 18-20hours (Figure 13).

The graft was then removed from the perfusion machine and transported in a sterile organ bag to the operating room. Due to the manipulation required over the perfusion period, the graft was bathed in betadine on a sterile back table prior to bringing the graft to the recipient. The graft was then flushed with heparinized saline to again confirm the outflow was present. Once this was confirmed, the graft implanted in the recipient. From a surgical standpoint, both recipient dissections were started in a similar way to the donor operation outlined earlier. Representative images from the recipient procedure are shown in Figure 15.

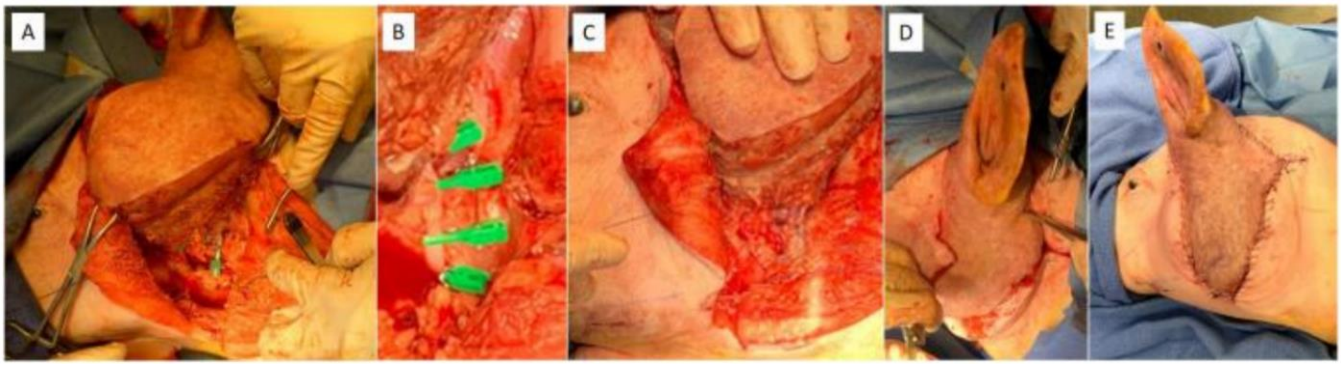


Figure 15: Hemifacial Flap Recipient Operation Images 3.3.21.

The skin was again marked in the same way and an incision was extended along the lateral neck, perpendicular to the caudal-most aspect of the skin marking posterior to the mandible (Figure 15A). Dissection was carried through the lateral neck incision to the sternocleidomastoid and paravertebral muscles with the goal of identifying the internal jugular vein (IJV) and carotid artery (CCA) (Figure 15B). The internal carotid artery (ICA) was dissected cranially until it bifurcated and dissected caudally to provide adequate length for anastomosis. The external jugular vein (EJV) was then identified coursing superficially to the SCM and isolated. The skin around the ear was then circumferentially incised as close to the base of the ear as possible. The skin around the ear was then undermined to create a skin flap. The ear was then transected leaving a rim of external auditory meatus cartilage which was used as the point of reattachment to the donor internal ear cartilage. The aspect of the skin incision coursing around the angle of the mandible was then extended ventrally to allow inset of the hemifacial graft. The parotid gland and masseteric fascia were dissected such that the donor masseter muscle could be inset (recipient dissection borders shown in Figure 15C, graft inset shown in Figure 15D-E). Graft EJV, graft IJV, and CCA were anastomosed to recipient EJV, recipient IJV, and recipient CCA respectively. The arteries were connected end-to-end in a sutured anastomosis. The venous anastomoses were accomplished with venous couplers (Figure 15C).

After the graft operation, a central line was placed in the pig for medication administration and access for labs. The final inset of the normothermically perfused graft is shown in Figure 15E. This pig (25762) was subsequently taken to the recovery area, where it was extubated, but shortly thereafter appeared to go into respiratory arrest from a cardiac etiology. Although compressions and life-saving drugs were administered with return of spontaneous circulation once,

the pig again arrested and was euthanized in recovery. Necropsy revealed pericardial, pleural, and abdominal effusions which were interpreted by the veterinary team to be related to a potential pre-existing cardiac condition. A connection to the normothermically perfused graft with tri-cavity effusions—typically a finding secondary to a chronic cardiac condition—is unclear at this time.

The other graft was triple bagged in sterile organ bags and stored at 4°C as a static cold storage control per Subtask 2 (Perform orthotopic facial transplantation using a swine leukocyte antigen (SLA) defined minipig model with major histocompatibility match with tacrolimus maintenance using grafts stored for 12 hours in static cold storage). For logistical reasons again, this was closer to a 23-hour static cold storage control. The graft was taken out of cold storage (Figure 16A), flushed, and inset into the second recipient (25761) as described above (Figure 16B-C). The final inset of the graft is shown in Figure 16C. Central line was placed in this pig as well. The pig was then taken to recovery and extubated without issue. Figure 16D shows the pig on POD 0 eating and appearing bright.

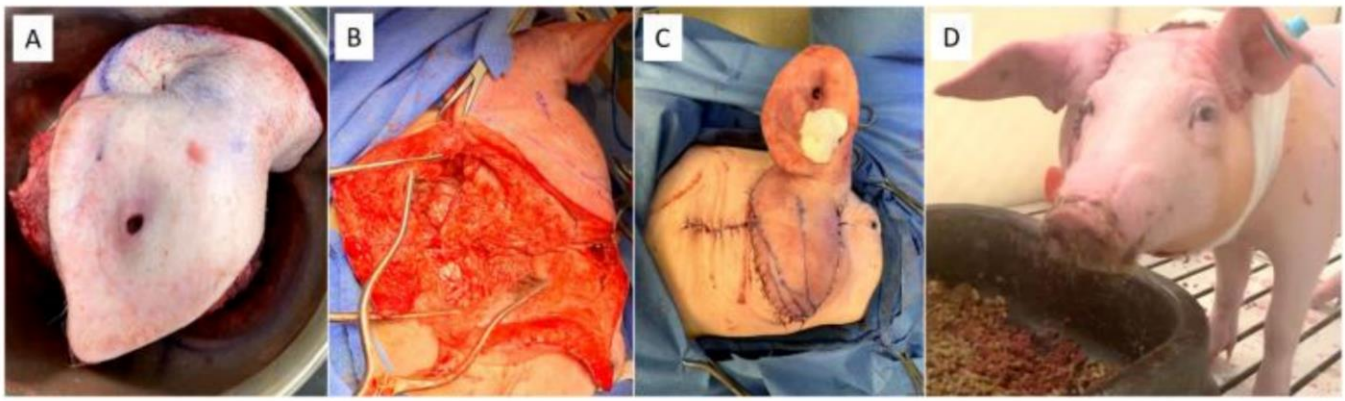


Figure 16: Hemifacial Flap SCS Control Graft Recipient 3.3.21.

This pig continued to recover well until POD 6, when significant peri-graft swelling was noted. The pig was sedated, and 150ml of straw-colored fluid was aspirated from the lateral neck consistent with seroma. Since then, this pig's post-operative course has been unremarkable and the pig is currently active, bright and eating well on POD 28. This pig's post-operative outcomes are quite encouraging and serve as a proof of concept for the surgical model underlying this project.

Following these milestones, several team meetings were had to discuss methods to ensure venous outflow throughout the prolonged perfusion period. As noted in the Site 1 achievements above, major advances to the perfusion system continue to be underway.

On 6/22, Site 2 harvested both hemifaces from a donor pig following preoperative cone beam CT-angiography to provide guidance on graft anatomy. Each hemiface was flushed with heparinized saline. The right hemiface was stored at 4 degrees C for 24 hours and gained modest mass during storage (459.2g to 513.8g; 112% increase). The left hemiface was perfused at physiologic temperature for 18 hours (Figure 17). The hemiface nearly doubled in mass during perfusion (406.0g to 775.4g; 191% increase). Perfusion was complicated by perfusate uptake by the graft, resulting in significantly decreased perfusate available for circulation. At this time, the graft demonstrated elevated potassium and lactate, as well as glucose depletion. At approximately 11 hours of perfusion, fresh perfusate was made and added to the system. Upon removal of the graft from the chamber, the ear was bright pink with two bullae lateral to the ear tag hole.

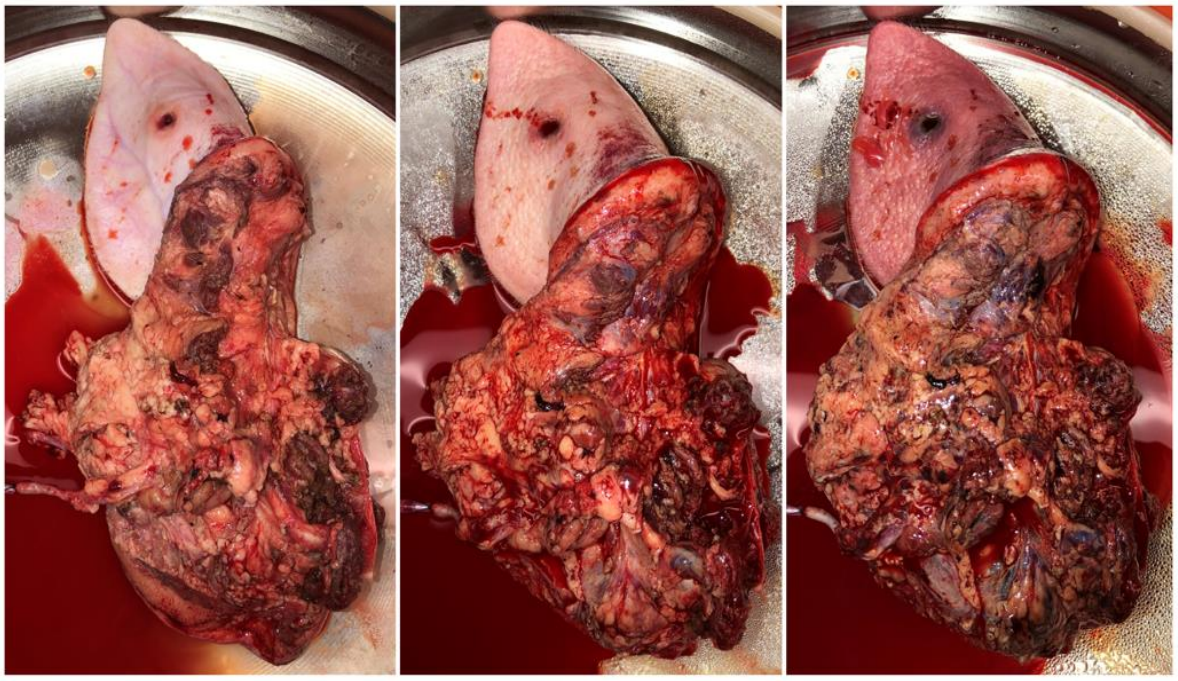
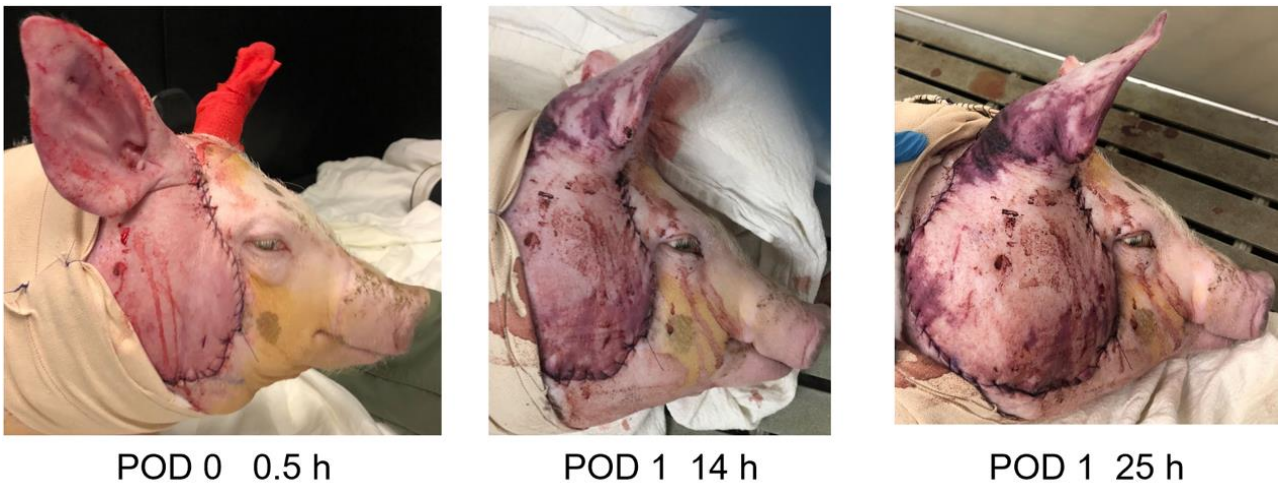


Figure 17: Hemifacial graft perfused at physiologic temperature. Left to right: Hemifacial flap at beginning of perfusion, 5.5 hours after perfusion, and 30 minutes before the end of perfusion.

On 6/23, Site 2 transplanted each hemiface onto a recipient pig following explant of the ipsilateral native hemiface. The recipient of the static cold storage hemiface recovered quickly from anesthesia, but the early postoperative course was complicated by progressive swelling of the graft and ipsilateral native face (Figure 18). Ultrasound of the graft and underlying tissues did not reveal a seroma or hematoma capable of drainage, and solumedrol is being used to combat swelling. The graft ear also demonstrates progressive sloughing of the superficial layer of skin (Figure 19).



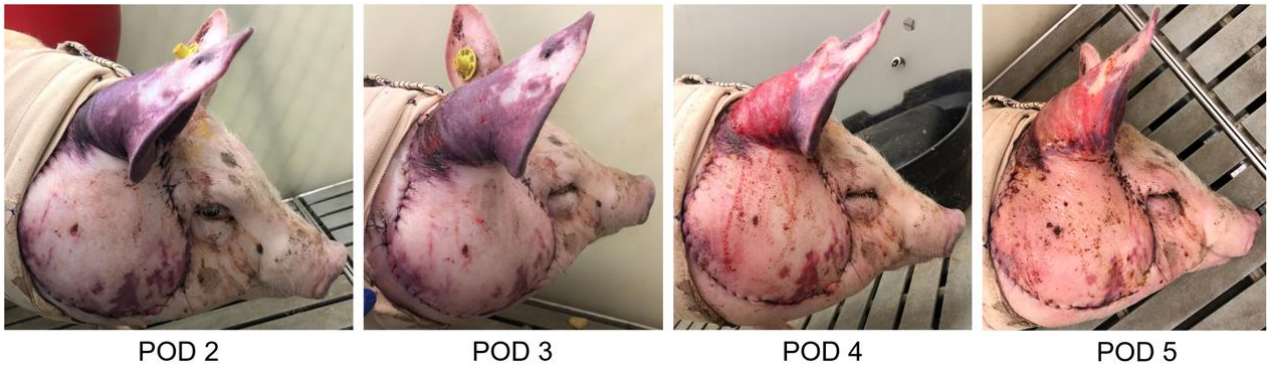


Figure 18: Progression of static cold storage hemiface following implantation.

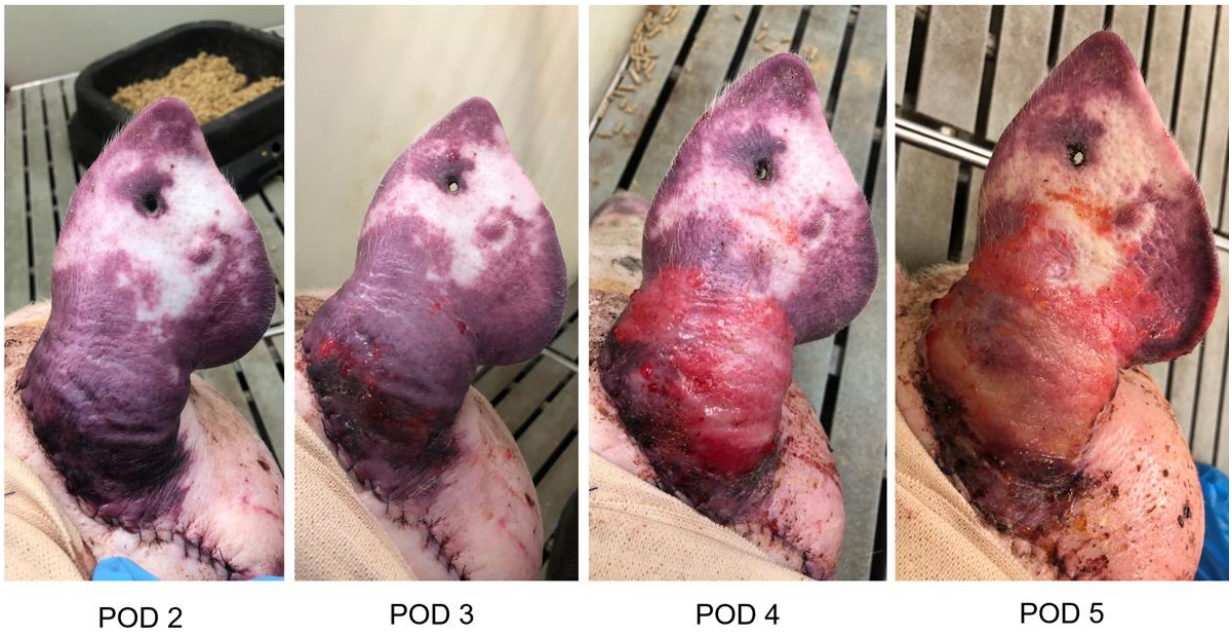


Figure 19: Progression of static cold storage related ear changes.

The recipient of the 18-hour physiologic temperature perfused hemiface demonstrated prolonged recovery time. This pig demonstrated less severe swelling compared with the aforementioned pig. However, the ear of the graft demonstrated progressive necrosis, beginning at the site of bullae first noticed in the perfusion chamber (Figure 20). The entire graft shifted from a bright purple to a dusky purple with darkening at the crease of the ear and edge of the graft (Figure 21). Discoloration and necrosis are likely due to vascular insufficiency of the graft. This pig is scheduled for euthanasia on 7/2.

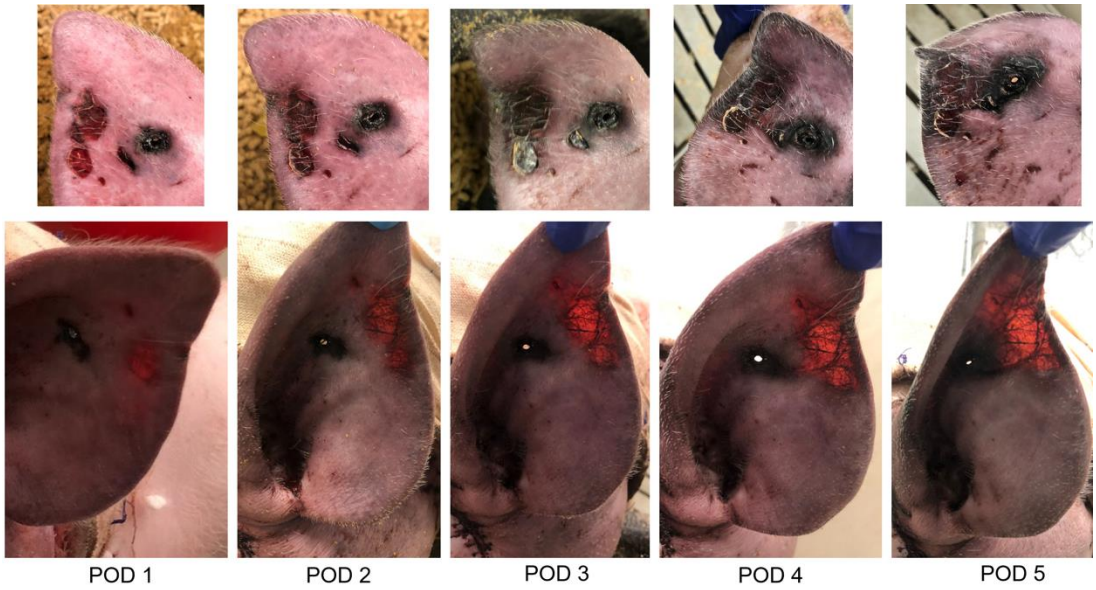


Figure 20: Progression of 18-hour machine perfused hemiface ear necrosis.

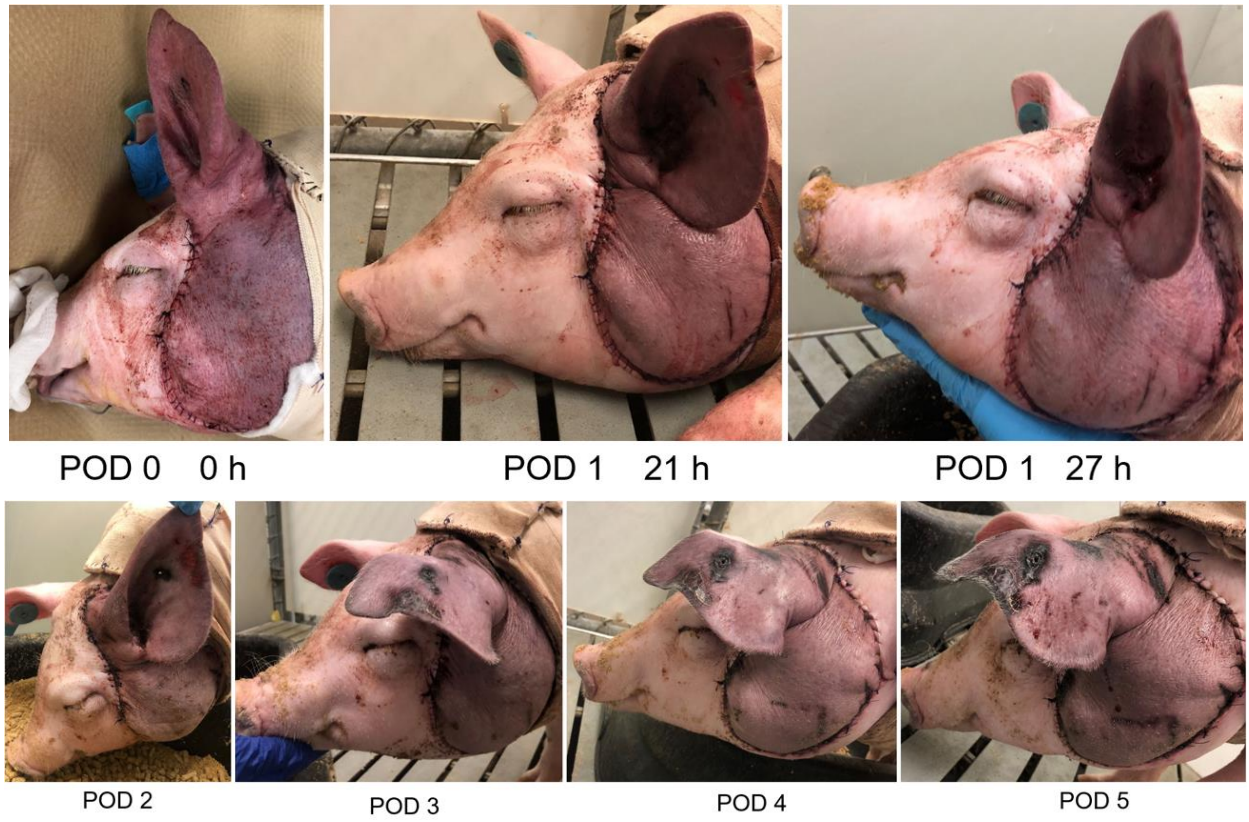
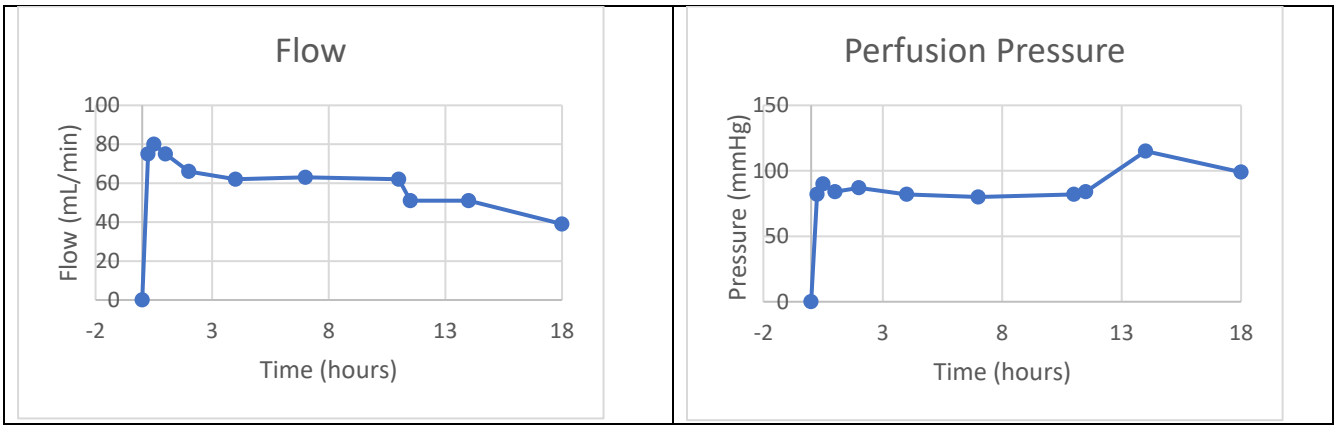


Figure 21: Progression of 18-hour machine perfused hemiface.

Under Major Task 1 Subtask 5, perfusion hemodynamics and chemistries were monitored for the flap over the 18-hour perfusion period (Figure 22). Hemodynamic data demonstrated titration of flap pressures a target of 80mmHg +/- 10mmHg. Average flow rate over the perfusion period was 56.5 ml/min. The graft demonstrated accumulation of lactate and potassium over time, as well as depletion of glucose. Additional perfusate was added to the system at 11.5 hours of perfusion. This corrected blood lab values, albeit temporarily. This suggests that an insufficient volume of perfusate was provided to the large graft at the onset of perfusion. Additionally, perfusion depletion was evidenced by the graft's accumulation of mass throughout the perfusion.



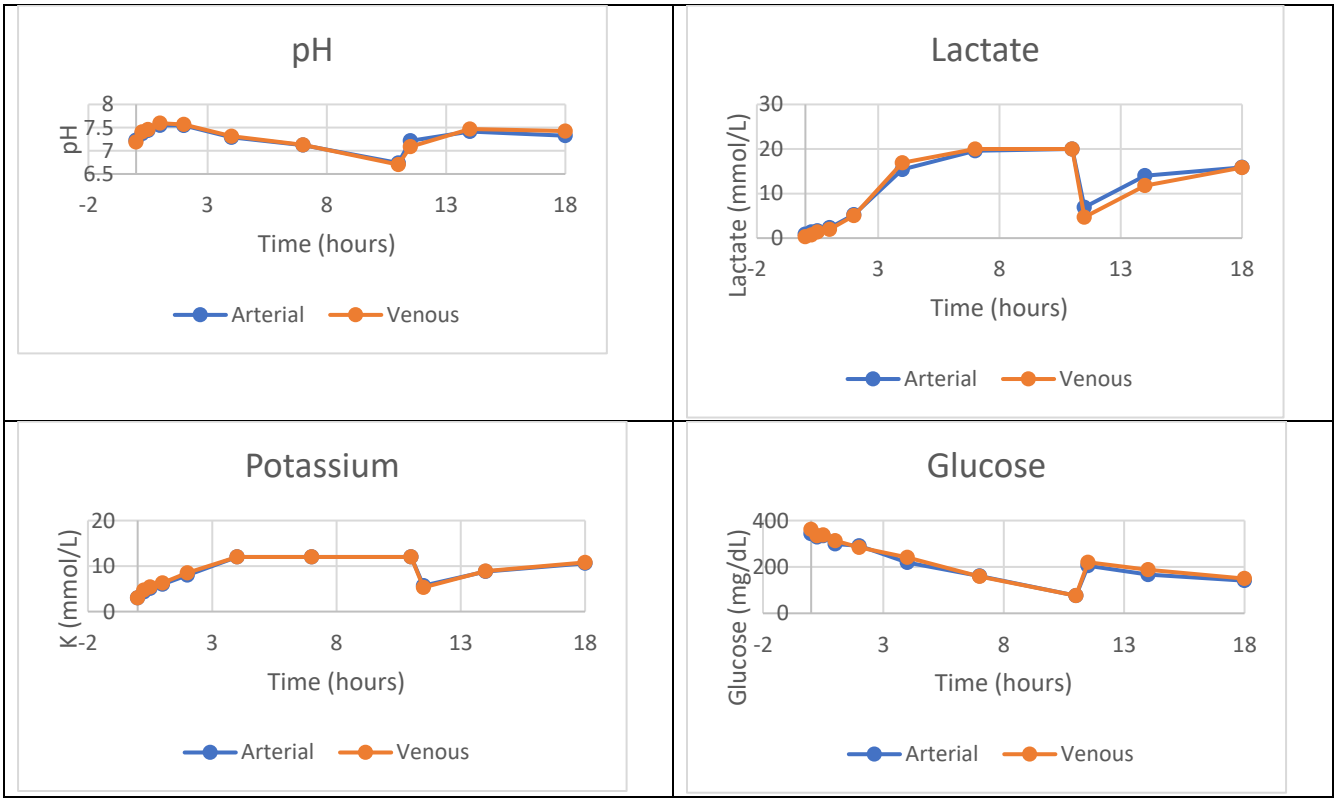


Figure 22: Perfusion Hemodynamics and Key Biological Parameters in an 18-hr Perfusion of Hemiface Explant at Physiologic Temperature (33.3°C-34.7°C)

- **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?**

  <u>Site 1:</u>

  In the upcoming third project year, Site 1 will complete the system development part of the workplan. This work will focus on refinements to the perfusion system and process. We will continue to address the special issues of face normothermic machine perfusion learned through our own work and published research.

  To accomplish the remaining workplan, we will complete two streams of work, 1) to fabricate and deliver a portable face preservation system by replicating the existing benchtop design as one that's lightweight and battery powered, and 2) to support the remaining perfusion studies in porcine transplant and human non-transplant models by maintaining, provisioning and refining the systems in step with the scientific work at Site 2.

  The portable system in the coming year will incorporate the refinements made to the benchtop systems this past year. Refinements include the disposable integrated organ holder and tubeset, electronics circuit boards, and manufactured wiring harness are lighter weight, smaller, and more reliable than their Year 1 predecessors. They can be used directly in the new portable system. Additionally, a battery and recharger circuit to provide power for the portable system, an overall layout of components for the portable system, and a risk analysis suitable for inclusion in the Design history were all completed in this past Year 2. Building on this foundation, the portable mainframe and enclosure will be completed in this Year 3, and the system verified and placed into use, perfusing a small series of human faces donated for research.

Our support responsibility for coming year's experimental work encompasses the provision of physical resources to accomplish machine perfusion on the remaining faces and hemifaces. These physical resources include perfusate and tubesets plus participation in studies and maintenance of the perfusion equipment. We will also provide hemodynamic analysis using data automatically collected by the perfusion machines and archived locally and via the cloud.

We are prepared to provide support responsive to the special factors of face perfusion learned in the course of this project. Cognizant of the established bounds and structure for this project, there may be new adaptations of the perfusion operating details that would benefit face viability at transplant. We anticipated some of these adaptations in the pitfalls section of our application, including the possible necessity for in-line disinfection and perfusate dialysis. For these two possibilities, we designed and tested modules to perform these functions, which we hold ready to implement, should data suggest we do. We also anticipate the possibility of swapping-out the perfusate after 12-hours to remove waste products en masse, which can be performed aseptically using our new module.

In practice, normothermic machine perfusion is beginning to incorporate advanced sensors that feed control loops of increasing sophistication to manage physical and chemical processes, including automatic dosing of drugs and substrates, transfusions, and electrochemical and gas balancing. These controls are all aimed at maintaining a regular physiologic environment for the vascularized structure being preserved. In this coming year, we will have the potential to incorporate additional sensors and infusion actuators into our perfusion system, including the capacity and bandwidth for robust data collection.

At the conclusion of this upcoming third project year, Site 1 will have provided and supported three perfusion systems at Site 2, comprising two benchtop systems and one portable system. The benchtop systems are in the process of upgrade, receiving new circuit board, onboard PC, new wiring harness, new disposable organ holder and integrated tubeset, and revised software. These upgrades will enhance reliability. An additional module for dialysis, plus two modules for disinfection are available if needed. Datalogging for hemodynamic analysis is included, accessible locally and via the cloud. The portable system is a new system to be provided in the first half of the coming project year, based on specifications developed by our work to date. We will continue to support the ongoing studies by maintaining and provisioning the systems, analyzing the data, making feasible refinements, and participating personally in experiments where needed.

Site 2:

Early progress included moving forward with the final graft harvest operation in Major Task one. Subtask 5 tasks were completed on samples from that day, and Subtask 6 review of data was performed with the surgical and engineering teams from Site 1 and Site 2 to ensure that the surgical and perfusion procedures were optimized for Major Task 2 transplant operations. Meetings were set with Site 2's ACUC veterinary team to ensure that post-operative swine care plans were clear for the Major Task 2 hemifacial transplant recipients. Surgeon and OR availability ere also been confirmed for Major Task 2 surgeries to allow progression through the SOW in a timely manner despite the COVID-19 shutdown.

After this, site one completed Major Task 1 Subtask 6 review of histopathology from Major Task 1's final harvest day and the first successful hemiface recipient was euthanized and samples collected and analyzed. In conjunction with site 2, procedures for subsequent hemifacial transplants were reviewed and optimized.

Subsequently, site two moved forward with the final graft harvest operation in Major Task one. Subtask 5 tasks were completed on samples from that day, and Subtask 6 review of data was performed with the surgical and engineering teams from Site 1 and Site 2 to ensure that the surgical and perfusion procedures had been optimized for Major Task 2 Transplant operations. Surgeon and OR availability was agaom confirmed for Major Task 2 surgeries to allow progression through the SOW in a timely manner despite the continued COVID-19 shutdown.

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**
  Nothing to report

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

Site 1:

Two benchtop systems have been at Site 2 for face perfusion since January 2020, with occasional absences for upgrades and repairs. The Site 1 organization, due to its small staff and midwestern location has been able to stay open and active throughout the pandemic to date, although it has faced the same shortages, delays, and price increases as experienced throughout industry. Mostly we have stayed on track towards achieving the project objectives, but with longer timelines, increased costs for purchasing, and additional effort for making substitutions. These activities became the bases for revised budget justification around injection molding tooling and possible revisions for an expected project fourth year.

Best estimate is that the Site 1 budget is currently 1 month overspent, and that delivery of the portable perfusion machine is approximately 2 months behind schedule. The explanation of budget status mainly reflects the higher than expected costs for injection molds, which has been described separately. The schedule status primarily relates to setbacks in the Site 2 schedule due to pandemic-related restrictions on activity at the hospital where that research is being performed. With remaining budget, we will complete the portable system and provide support for the experimental program through the three-year project timeframe.

However, indications are that the project will likely continue experimentally for a fourth year at Site 2. Our plans would be to continue to support the project, including provision of our benchtop perfusion machines plus experimental involvement, whether as invoiceable work or self-funded. This possibility has been also the subject of separate correspondence.

Interestingly, we are at the stage of the experiments and project that we are generating essential first-ever data on normothermic face perfusion. Our whole team expects process and outcomes discoveries. Based on research with other organs and structures we have prepared dialysis, disinfection, and perfusate swapping modules to help alleviate the most expectable problems. We have also installed a robust data gathering capability within each benchtop system that will enable time-stamped post hoc analysis of all sensors and controls readouts at 1 hertz or greater. To support data gathering, we have obtained blood gas sensors to add to the system if needed. Collaborating teams at both Sites 1 and 2 look forward to solving these problems and performing focused optimizations, as we are positioned by our system development and research work to address them.

Site 2:

At the beginning of this annual reporting cycle, Site 2 was recently notified that Columbia University had signed a new agreement with Choironex to manage their swine herd and breeding colony. As part of this change, they relocated the herd to a new facility in upstate New York. While this did not lead to a disruption in animal supply, the cost of each animal had been significantly increased to $3,500 (rather than $2500) effective immediately. This obviously impacted expenditures and Site 2 spent considerable time and effort pursuing the possibility of using alternative swine sources. Initiation of discussions with the grant officer was performed and a plan went underway to allow a smooth transition to a potential alternative swine species.

In terms of progress, site two moved forward with the final graft harvest operation in Major Task one. Subtask 5 tasks as be completed on samples from that day, and Subtask 6 review of data will be performed with the surgical and engineering teams from Site 1 and Site 2 to ensure that the surgical and perfusion procedures have been

optimized for Major Task 2 Transplant operations. A meeting is also being scheduled with Site 2's ACUC veterinary team to ensure that post-operative swine care plans are clear for the Major Task 2 hemifacial transplant recipients. Surgeon and OR availability has also been confirmed for Major Task 2 surgeries to allow progression through the SOW in a timely manner despite the impacts of the COVID-19 pandemic.

- **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**
  Nothing to report

- **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

Site 1:

Two existing benchtop perfusion machines have been revised to enable the normothermic perfusion of porcine hemifaces for the aims of this project.

Site 2:

Nothing to report.

7. Participants & Other Collaborating Organizations

- **What individuals have worked on the project?**

Name:	John Brassil
Project Role:	Principal Investigator
Nearest person month worked:	9
Contribution:	Mr. Brassil manages the Site 1 project including the scientific, business and engineering activities.
Name:	Gerald Brandacher, M.D.
Project Role:	Principal Investigator
Nearest person month worked:	1
Contribution:	Dr. Brandacher has supervised all animal surgeries and discussions to move this project forward.
Name:	Byoung Chol Oh, D.V.M., Ph.D.
Project Role:	Co-Investigator
Nearest person month worked:	1
Contribution:	Dr. Oh has been involved in all animal surgeries, post-surgical data analysis and processes, as well as discussions to move this project forward.
Name:	Damon Cooney M.D., Ph.D.
Project Role:	Co-Investigator
Nearest person month worked:	1
Contribution:	

	Dr. Cooney has worked to establish and complete the IACUC protocol. He has made important contributions to surgical planning for the proposed model.
Name: Project Role: Nearest person month worked: Contribution:	Giorgio Raimondi Ph.D. Co-Investigator 1 Dr. Raimondi has made contributions to data analysis for the proposed model.
Name: Project Role: Nearest person month worked: Contribution:	Richa Kalsi, M.D. Research Fellow 6 (ended 06/30/2021) Dr. Kalsi is involved in all animal surgeries, post-surgical data analysis and processes, as well as discussions to further project refinement and optimization.
Name: Project Role: Nearest person month worked: Contribution:	Christopher D. Lopez, M.D. Research Fellow 6 (Started 07/01/2021) Dr. Lopez is involved in all animal surgeries, post-surgical data analysis and processes, as well as discussions to further project refinement and optimization.
Name: Project Role: Nearest person month worked: Contribution:	Angela Estevez Technician 3 Ms. Estevez has provided technical and administrative assistance for this project

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Gerald Brandacher	Change: Ended - Phase II: Non-Toxic, Highly-Effective Bioinspired Cryoprotectants Role: PI Effort: 1% Date: 11/01/2019 – 10/31/2020
Gerald Brandacher	Change: Received - 2R44AI124835 - High Subzero Equilibrium VCA Cryopreservation Role: PI Effort: 1% Date: 04/01/2020 – 12/31/2021
Gerald Brandacher	Change: Received – 2020-MSCRFL-5414 Human iPSC-derived EGFR+ functional Schwann Cells to Enhance Nerve Regeneration Role: Co-I Effort: 2% Date: 06/30/2020 – 06/29/2022
Gerald Brandacher	Change: Received – 5527 - Assessing the Comparative and Longitudinal Benefits of Vascularized Composite Role: PI Effort: 1% Date: 09/30/2020 – 09/29/2022
Gerald Brandacher	Change: Received - R43HL152941- Feasibility of expanding ischemia time for hearts destined for transplantation Role: PI Effort: 1% Date: 01/01/2021 – 08/31/2022
Gerald Brandacher	Change: Received - 1R43Al155196 - A Novel and Clinically Feasible Co-therapy of Deceased Donor Bone Marrow Combine Role: PI Effort: 3% Date: 02/15/2021 – 06/30/2022
Gerald Brandacher	Change: Received - Replacing Sutures for Microvascular and Vascular Anastomosis Role: Co-I Effort: 1%

	Date: 01/13/2021 – 12/13/2021
Gerald Brandacher	Changes: Received – "C00069632-2 Quantitative Ambulatory Assessment and Prognosis of the Impact of Severe Upper…" Role: PI Effort: 2% Dates: 03/01/2021 – 02/29/2024

- **Actual Problems or delays and actions to resolve them**

  Site 1 and Site 2:

  Injection molding tools originally budgeted at $62,820 are now expected to cost $106,755. This increase in cost is primarily based on the increase in the bioreactor and lid diameter for 207 mm to 360 mm. Costs for materials and supplies originally budgeted at $90,763 are now projected at $149,498. This increase includes the cost of a biochemistry analyzer, the subject of a previous contract modification. Other unplanned costs attributed to equipment and supplies include additional revision and improvements to the perfusion system, including the revised electronics of the current reporting period, plus higher than budgeted shipping costs related to the pandemic. Notwithstanding these category excesses, the project is expected to meet the original $620,409 budget within the original 3-year time frame as compensating savings have been achieved in employee fringes, and consulting savings due to cancellation of the consultant work in Belgium.

- **Anticipated Problems/Issues**

  There are no new anticipated problems or delays. Pandemic related delays have affected the animal surgical schedule and the turn around time by some key vendors. These delays were noted in the Annual Report and remain unchanged.

- **What other organizations were involved as partners?**
  Nothing to report

8. Special Reporting Requirements

QUAD CHART: Submitted with attachments.