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TITLE: Heparin-Free Extracorporeal Life Support for Point-of-Need Treatment of Single and Multiorgan Failure

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CONTRACTING ORGANIZATION: The Geneva Foundation

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14. ABSTRACT

In this award we will develop and test a next generation ECLS system without systemic heparin administration using a combination of slippery surface blood repellent coating and impregnation of the ECLS circuitry with an extended release nitric oxide (NO) formulation. A translational team composed of clinician scientists, chemists, respiratory therapists, and biomedical engineers at The Geneva Foundation, USAISR and the University of Georgia (UGA), will carry out a series of experiments. Rationale: the most severely injured combat casualties require new interventions which permit lung rest and recovery and support and stabilize vital functions during prolonged field care (PFC) and aeromedical evacuation. ECLS is life-saving in the most severely injured in whom mechanical ventilation (MV) failed but requires systemic heparinization which is contraindicated in trauma. Objective/Hypothesis. Our research objective is to develop a new treatment for combat casualties with ARDS using ECLS without systemic heparinization. Hypothesis: Liquid-infused, nitric oxide releasing (LINOREL) coating permits heparin free ECLS in trauma while improving lung function, reducing minute ventilation and providing sustainable multiorgan support during PFC with aeromedical evacuation at altitude.

15. SUBJECT TERMS
ARDS ; Multi-organ failure ; ECLS ; prolonged field care; nitric oxide; coagulation; blood compatibility; biomaterials

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1. **INTRODUCTION:** This project addresses the 2018 JWMRP research priorities by developing a new point of need treatment and device which will reduce the incidence and severity of acute respiratory distress syndrome (ARDS) and/or multiorgan failure secondary to trauma, transfusion, burns, hemorrhagic shock. This project was inspired by and is a logical continuation of the "Transportable Life Support for Treatment of Acute Lung Failure Due to Smoke Inhalation and Burns", W81XWH-13-2-0006 award which introduced feasibility of minimally invasive extracorporeal life support (ECLS) as an early intervention in combat but requires heparin administration. In this award we will develop and test a next generation ECLS system without systemic heparin administration using a combination of slippery surface blood repellent coating and impregnation of the ECLS circuitry with an extended release nitric oxide (NO) formulation. A translational team composed of clinician scientists, chemists, respiratory therapists, and biomedical engineers at The Geneva Foundation, USAISR and the University of Georgia (UGA), will carry out a series of experiments. Rationale: the most severely injured combat casualties require new interventions which permit lung rest and recovery and support and stabilize vital functions during prolonged field care (PFC) and aeromedical evacuation. ECLS is life-saving in the most severely injured in whom mechanical ventilation (MV) failed but requires systemic heparinization which is contraindicated in trauma. Objective/Hypothesis. Our research objective is to develop a new treatment for combat casualties with ARDS using ECLS without systemic heparinization. Hypothesis: Liquid-infused, nitric oxide releasing (LINOREL) coating permits heparin free ECLS in trauma while improving lung function, reducing minute ventilation and providing sustainable multiorgan support during PFC with aeromedical evacuation at altitude.

2. **KEYWORDS:**

ARDS; Multi-organ failure; ECLS; prolonged field care; nitric oxide; coagulation; blood compatibility; biomaterials

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Specific Aim 1: Develop and optimize the combination of the biofouling-resistant silicone liquid-infused surfaces with the NO-releasing polymer using S-nitroso-N-acetylpenicillamine (SNAP).	**Timeline (Months)**	**Site 1 Batchinsky AREVA**	**Site 2 Handa UGA**	**Percentage Complete:**
Subtask 1: Conduct characterization of the combined liquid-infused/NO-releasing surfaces.	1-10	Dr. Batchinsky	Dr. Handa	
Objective 1a: Incubate combined coatings for up to 1 week, periodically testing for NO release	1-6		Dr. Handa	100%
Objective 1b: Identify which combinations of SNAP produce stable release over 7 days	1-10		Dr. Handa	100%
MILESTONE: Identify candidate combination of impregnation/coating to move forward to Aim 2	1-10		Dr. Handa	100%
Subtask 2: Assess the bacteriostatic and antibacterial properties of LINOREL	1-10	Dr. Batchinsky	Dr. Handa	
Objective 2a: Assess antibacterial properties of candidate LINOREL coating *in vitro.*	1-10		Dr. Handa	100%
MILESTONE: Identify biofilm reduction properties of candidate LINOREL coating	1-10		Dr. Handa	100%
Subtask 3: *In vivo* evaluation of the liquid-infused/NO polymer coating in small scale ECC rabbit model.	11-18	Dr. Batchinsky	Dr. Handa 40 rabbits	
Objective 3a: Write animal use protocol, receive regulatory approvals	11	Dr. Batchinsky	Dr. Handa	100%
Objective 3b: Experimental group 1: control silicone tubing with no additives **(n=10)**	11-18		Dr. Handa	90%
Objective 3c: Experimental group 2: NO release only **(n=10)**	11-18		Dr. Handa	90%
Objective 3d: Experimental group 3: Liquid infused only **(n=10)**	11-18		Dr. Handa	90%
Objective 3e: Experimental group 4: Candidate LINOREL **(n=10)**	11-18		Dr. Handa	90%

MILESTONE: Receive regulatory approvals for animal use	11	Dr. Batchinsky	Dr. Handa	100%
MILESTONE: Complete rabbit model of ECC	11-18	Dr. Batchinsky	Dr. Handa	90%
Subtask 4: *In vitro* evaluation of the liquid-infused/NO polymer coating in full-scale ECLS ex vivo set-up with porcine donor blood.	11-18	Dr. Batchinsky		
Objective 4a: Write laboratory research protocol	11	Dr. Batchinsky		100%
Objective 4b: Experimental group 1: control silicone tubing with no additives **(n=10)**	11-18	Dr. Batchinsky		90%
Objective 4c: Experimental group 2: NO release only **(n=10)**	11-18	Dr. Batchinsky		90%
Objective 4d: Experimental group 3: Liquid infused only **(n=10)**	11-18	Dr. Batchinsky		90%
Objective 4e: Experimental group 4: Candidate LINOREL **(n=10)**	11-18	Dr. Batchinsky		90%
MILESTONE: Complete ex vivo ECLS model	11-18	Dr. Batchinsky	Dr. Handa	85%
Specific Aim 2: Determine device performance and systemic effects of LINOREL-coated ECLS circuitry in 72-hour in vivo experiment in healthy swine.	12-24	Dr. Batchinsky		
Subtask 5: *In vivo* evaluation of the liquid-infused/NO polymer coating in **uninjured** swine	12-24	Dr. Batchinsky 18 swine	Dr. Handa	
Objective 5a: Write animal use protocol, receive regulatory approvals	12	Dr. Batchinsky	Dr. Handa	100%
Objective 5b: Experimental group 1: ECLS with manufacturer's standard circuit (with immobilized heparin coating) and continuous heparinization **(n=9)**	12-18	Dr. Batchinsky	Dr. Handa	0%
Objective 5c: Experimental group 2: ECLS with LINOREL combination coating without systemic heparinization **(n=9)**	18-24	Dr. Batchinsky	Dr. Handa	0%
Specific Aim 3: Evaluate the use of LINOREL-coated ECLS without systemic heparin in vivo in a combat relevant aeromedical evacuation ARDS model.	18-28	Dr. Batchinsky	Dr. Handa	
Subtask 6: *In vivo* evaluation of the liquid-infused/NO polymer coating in **injured** swine	18-28	Dr. Batchinsky 18 swine	Dr. Handa	
Objective 6a: Write animal use protocol, receive regulatory approvals	18	Dr. Batchinsky		100%
Objective 6b: Experimental group 1: ECLS with manufacturer's standard circuit (with immobilized heparin coating) and continuous heparinization **(n=9)**	18-28	Dr. Batchinsky		0%
Objective 6c: Experimental group 4: ECLS with LINOREL combination coating without systemic heparinization **(n=9)**	24-28	Dr. Batchinsky		0%
MILESTONE: Receive regulatory approvals for injured animal use	18	Dr. Batchinsky		50%
MILESTONE: Complete 72 hour injured swine models	28	Dr. Batchinsky		5%
Specific Aim 4: Evaluate the efficacy, functionality and stability of LINOREL-coated ECLS materials following 72-hours of in vivo testing.	30-36	Dr. Batchinsky	Dr. Handa	0%
Subtask 7: Assess coating stability after 72hr testing.	30-36	Dr. Batchinsky	Dr. Handa	
Objective 7a: Circuit components and tubing samples will be analyzed by Batchinsky lab and Handa lab for stability and functionality after 72hr use.	12-36	Dr. Batchinsky	Dr. Handa	0%
MILESTONE: Complete post-explantation SEM analysis of membranes after *in vivo* use	36	Dr. Batchinsky	Dr. Handa	0%
Specific Aim 5: Prepare pre-FDA validation data collection using GLP in 72h in vivo model of heparin-free LINOREL ECLS	36-46	Dr. Batchinsky		0%
Subtask 8: *In vivo* evaluation of the liquid-infused/NO polymer coating in swine	34-46	Dr. Batchinsky 12 swine		
Objective 8a: Write animal use protocol, receive regulatory approvals	34	Dr. Batchinsky		0%
Objective 8b: Experimental group: ECLS with LINOREL combination coating without systemic heparinization **(n=12)**	34-46	Dr. Batchinsky		0%
MILESTONE: Receive regulatory approvals for injured animal use	34	Dr. Batchinsky		0%

MILESTONE: Complete 72 hour injured swine models under GLP standards	34-46	Dr. Batchinsky		0%
MILESTONE: Complete and submit pre-FDA validation data package	34-46	Dr. Batchinsky		0%
MILESTONE: Complete final reports and manuscripts	46-48	Dr. Batchinsky		0%

What was accomplished under these goals?

Major Activity: Develop and optimize the combination of the biofouling-resistant silicone liquid-infused surfaces with the NO-releasing polymer using S-nitroso-N-acetylpenicillamine (SNAP).

I. Subtask 1: Conduct characterization of the combined liquid infused/NO-releasing surfaces.

-Subtask 1 completed in Y1, please see Y1 Annual Report for findings and conclusions.

Additional study performed in Y2 to assess protein adhesion:

a) Results/Findings/Developments: See **Figure 1**. Levels of protein adhesion were quantified for fabricated sponge coatings using a modified version of a previously reported method[1]. FITC labeled human fibrinogen was diluted with unlabeled fibrinogen solution to achieve a 1:10 ratio of 4 mg mL^{-1} fibrinogen in phosphate buffer solution (pH 7.4). Sections of the various sponge samples were incubated in phosphate buffer solution at 37 °C for 30 minutes in a 96 well plate, followed by the addition of the physiological fibrinogen solution to achieve a final concentration of 2 mg mL^{-1}. After 90 minutes of incubation in the protein solution, samples were infinitely diluted in order to wash away any loosely bound protein from the sponges. Adsorbed fibrinogen was quantified by measuring the excitation/emission of sample at 495/519 nm and interpolated using a standard curve of FITC labeled fibrinogen with a 1:10 dilution factor.

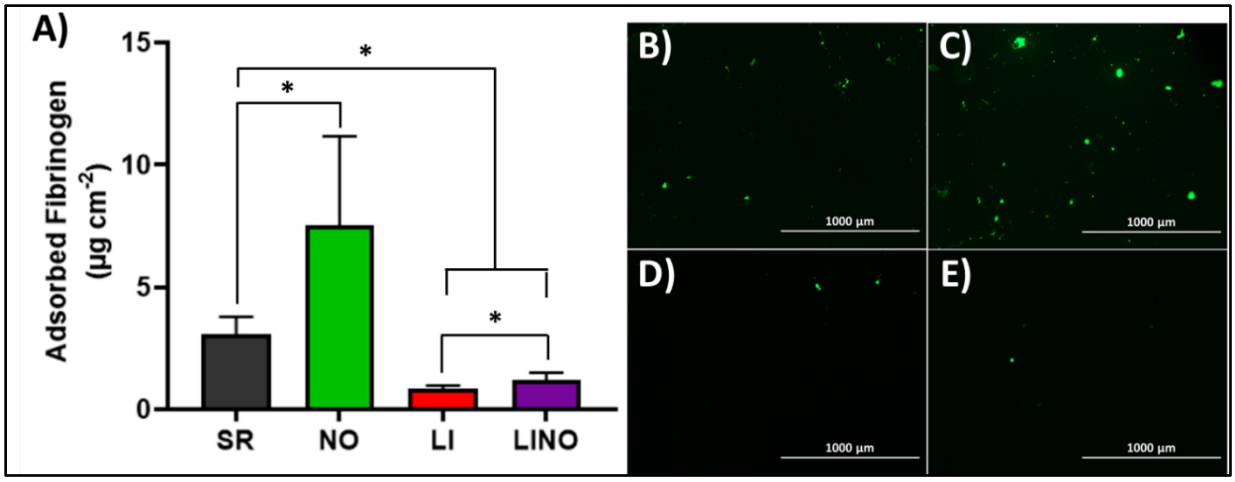

Figure 1- Reduction of fibrinogen adsorbed on silicone tubing substrates after 1.5 h of incubation. **A)** Quantitative evaluation using fluorescent intensity and a calibration curve. **B-E)** Qualitative imaging of fluorescently labeled fibrinogen of **B)** Control SR **C)** NO-releasing SR **D)** Liquid-Infused SR **E)** LINOREL SR. Data represents mean ± standard deviation. Statistical significance is indicated by * ($p \leq 0.05$).

b) Conclusions/Achievements: Control SR exhibited a fibrinogen adsorption of 3.06 ± 0.72 µg cm^{-2}, which was increased by 140% to 7.51 ± 3.66 µg cm^{-2} in NO-releasing samples. The inclusion of silicone oil reduced fibrinogen adsorption to 0.85 ± 0.11 µg cm^{-2} on LI samples (a 72.20% reduction) and 1.19 ± 0.31 µg cm^{-2} on LINOREL samples (a 61.29% reduction).

II. Subtask 2: Assess the bacteriostatic and antibacterial properties of LiNORel.

A. Assess antibacterial properties of candidate LiNORel coating *in vitro*.

a) Results/Findings/Developments: *In vitro* bacterial adhesion analysis was conducted via a continuous flow CDC bioreactor. The polymer samples were challenged with Gram-positive *Staphylococcus aureus (S. aureus)* and *Staphylococcus epidermidis (S. epidermidis)*, Gram-

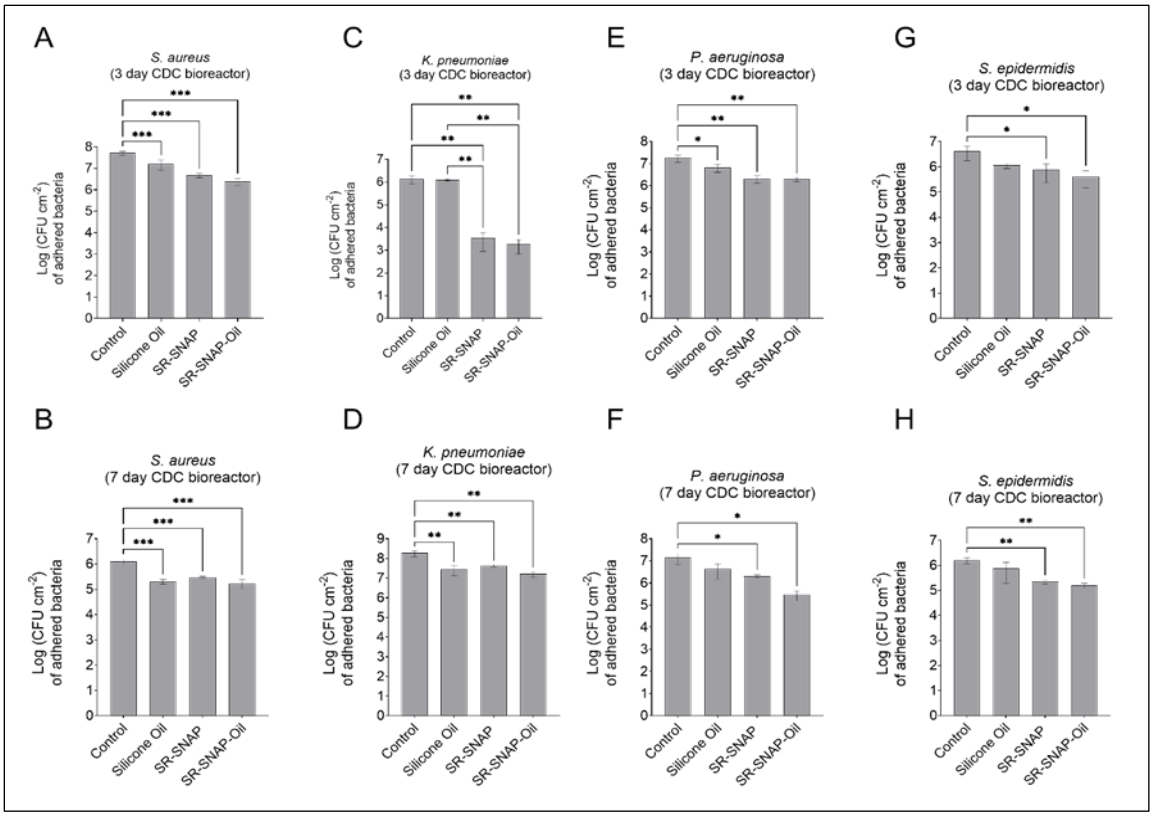

Figure 2 - Reduction of viable adhered bacteria on silicone tubing substrates upon 3 days (A, C, E, G) and 7 days (B, D, F, H) exposure to *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *S. epidermidis*. Data represents mean ± standard deviation. Statistical significance is indicated by * ($p \leq 0.05$), **($p \leq 0.01$), ***($p \leq 0.001$)

negative *Pseudomonas aeruginosa (P. aeruginosa)* & *Klebsiella pneumoniae* (*K. pneumoniae*) for 3 days and 7 days. Bacterial solution at a concentration range of $10^5 – 10^6$ was prepared in 2g/L LB broth. The samples were incubated with 300 mL (at 37°C, 200 rpm) of the bacterial solution inside the CDC bioreactor setup for 90 mins before starting the continuous flow phase at a flow rate of 1.6 mL min^{-1} (at 37°C, 120 rpm). After 3 and 7 days, the samples were removed and gently washed with 0.01 M PBS to remove any loosely attached or planktonic bacteria. The samples were then homogenized at 25000 rpm for 1 min. The homogenization step detaches adhered bacteria from the polymer samples into the PBS buffer. The bacteria-PBS solution was serially diluted and plated on LB agar to enumerate colony forming units (CFU). The bacterial concentration is represented as CFU per cm^2 of the polymer samples. **Figure 2** demonstrates the reduction in viable adhered bacteria upon polymer samples – Control (Silicone rubber/SR), Silicone oil, SR-SNAP (NO Donor), SR-SNAP-Silicone oil (LiNORel) over 3 days and 7 days of continuous flow conditions achieved through a CDC bioreactor. The result shows a general trend of decreased viable adhered bacteria for SR-SNAP-Silicone oil. In the past, exposure of *P. aeruginosa* and *S. aureus* upon LiNORel surfaces have demonstrated 88 ± 1.0 % and 99 ± 0.1 % reduction efficiency for 7 day- CDC.[2] The current study shows a similar trend with reduction efficiency of 98 ± 1.2 % and 87 ± 6.4% with *P. aeruginosa* and *S. aureus* when challenged against LiNORel surface for 7 days. *S. epidermidis* and *K. pneumoniae* were evaluated against LiNORel for the first time in this study. The reduction efficiency after 7-day exposure against *S. epidermidis* and *K. pneumoniae* were 90.34 ± 1.2 % and 91.26 ± 3.6%, respectively. Reduction efficiency from 3-day studies of *P. aeruginosa* (89.2 ± 2.7 %), *S. epidermidis* (89.9 ± 9.2%), *S. aureus* (95.4 ± 2%) and *K. pneumoniae* (99.8 ± 0.09%) reflect similar trend of bacterial suppression where SR-SNAP-Oil outperforms control tubings. The results suggest, the combined and synergistic effect of silicone oil surface and NO release from the bulk polymer matrix can successfully prevent bacterial adhesion and thereby reducing chances of infection.

b) Conclusions/Achievements: Compared to Control/uncoated silicone rubber (SR), LiNORel coating (SR-SNAP-Oil) significantly reduced adhesion of all bacterial species tested.

III. Subtask 3: In vivo assessment of LiNORel coating in small scale rabbit extracorporeal circulation model.

A. Objective 1a: Write animal use protocol, receive regulatory approval

a) Results/Findings/Developments: The original IACUC protocol for this study at UGA expired on 18 Jan 21. PI Dr. Handa received IACUC approval for the new protocol on 24 Dec 20. Dr. Handa was notified by ACURO on 18 Jan 21 that they did not have a record of approval for the new/non-expired protocol. Dr. Handa submitted the new IACUC-approved protocol to ACURO on 18 Jan 21, and ceased all studies awaiting response from ACURO. ACURO responded on 19 Feb 21 stating they had not received the new protocol. On 22 Jan, ACURO clarified that the documents had been received on 18 Jan 21, but that the documents needed to be corrected. Dr. Handa submitted the corrected documents and received approval from ACURO on 08 April 21 for the renewed protocol.

Custom extracorporeal circulation tubing segments (ECCs) were prepared at UGA. Briefly, the circuits were constructed consisting of 16-gauge (arterial) and 14-gauge (venous) IV polyurethane angiocatheters, two 16 cm lengths of 1/4 inch inner diameter silicone tubing, and one 8 cm length of 3/8 inch silicone tubing to create a thrombogenicity chamber to promote stagnant and recirculating regions of blood flow. The angiocatheters were coated thrice with a dilute solution of ChronoSil (a silicone elastomer) in tetrahydrofuran (20 mg mL^{-1}) with or without 10% weight S-nitroso-N-acetylpenicillamine (SNAP). All ECC loops segments were adhered together using a commercial silicone adhesive. The ECC loops were thereafter dried under ambient conditions overnight to allow the silicone adhesive to dry and the tetrahydrofuran to evaporate. NO and LiNORel loops had their tubing segments swelled in a solution of 25 mg/mL SNAP in tetrahydrofuran before ECC construction, and liquid-infused and LiNORel samples were primed with silicone oil after ECC construction to induce. Prior to ECC placement, the ECC loops were primed with saline solution for 30 mins, drained, and fresh saline priming solution was added immediately before insertion to prevent any systemic delivery of NO from any SNAP that may have leached during the initial incubation period.

Anesthetized New Zealand white rabbits (2.5-3.5 kg) were anesthetized and maintained on anesthesia throughout the 4-hour circulation study. Prior to placement of the ECC, the rabbit left carotid artery and right external jugular vein were isolated. After baseline blood measurements, the ECC was then placed into position by cannulating the left carotid artery for ECC inflow and the right external jugular vein for ECC outflow. The flow through the ECC was started by unclamping the arterial and venous sides of ECC and blood flow in circuit will be monitored with an ultrasonic flow probe and flow meter. Animals were not systemically anticoagulated during the experiments. Additional blood samples were drawn hourly to observe blood function and composition over time. After 4 h of flow, the circuits were removed from animal, rinsed with 60 mL of saline and drained. Gross analysis of the ECC loop was performed. Images were taken down the lumen of the thrombochamber to assess patency, and the thrombochamber was cut in half to assess total surface area of thrombus formation using imaging technology. Thereafter, the thrombus was removed, weighed, and then preserved in a 10% formalin solution. Parts of the smaller diameter tubing were cut and preserved for SEM imagining by placing in 2% glutaraldehyde solution. NO-releasing samples had post-ECC NO release assessed, and slippery samples had their post-ECC sliding angle assessed.

Current data supports the notion that the LINOREL tubing improves *in vivo* hemocompatibility. Of the n=6 ECCs tested, all NO, LI, and LINOREL ECCs were able to flow for the 4 h period. However, 2 out of the 6 control SR ECCs clotted within the 4 hour period (at ≈1.5 h and ≈ 2.5 h). As shown in **Figure 3,** the LINOREL ECCs were able to significantly reduce both the thrombus mass and surface area of thrombus formation in the ECC thrombochamber. In addition, NO, LI, and LINOREL samples showed a significant improvement of platelet consumption over the 4 h of blood flow by maintaining ≥ 90% baseline platelet consumption (**Figure 4)**. While there was no statistically significant difference, LINOREL samples had a lower percentage white blood count (WBC) after 4 h than all other sample types; and NO, LI, and LINOREL samples showed no effect on the ability of platelets to aggregate over the 4 h time span. Furthermore, NO, LI, and LINOREL samples showed superior flow rates over the 4 h period with no difference in mean arterial pressure or heart rate (**Table 1**). Additional data, such as flow cytometry and coagulation studies, are still being analyzed and will be included in the next report and further statistical studies will be conducted to assess synergistic behaviors.

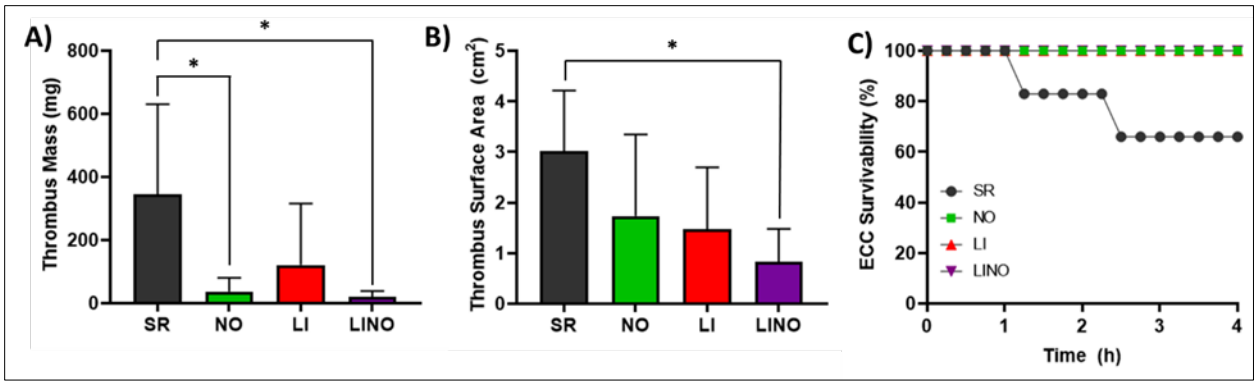

Figure 3- A) Thrombus mass in the ECC thrombochamber. **B)** Surface area of thrombus formation in the ECC thrombochamber, which was quantified by software imaging. **C)** ECC survivability over the 4 h period. Data represents mean ± standard deviation (n=6). Statistical significance is indicated by * ($p \leq 0.05$).

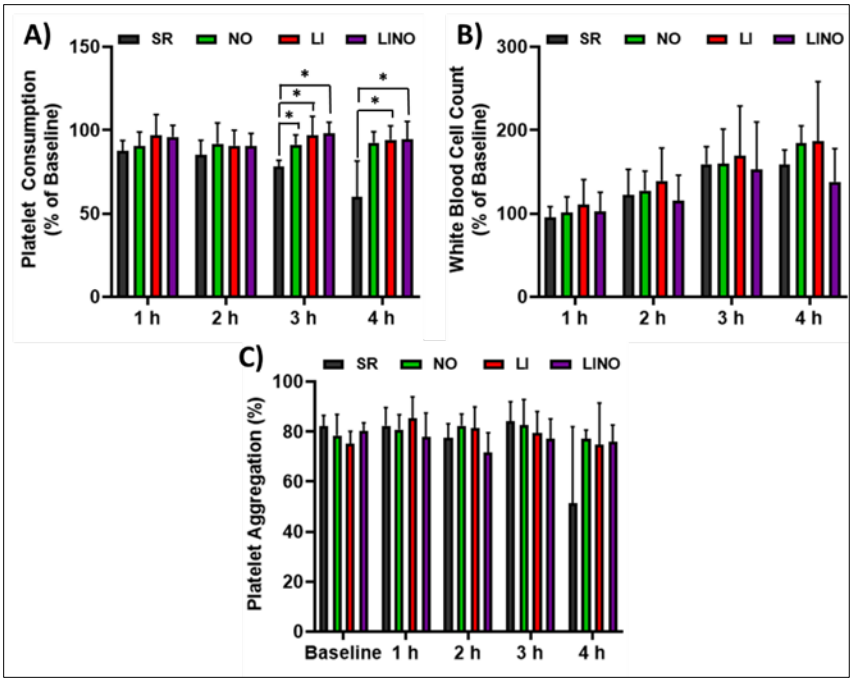

Figure 4: A) Thrombus mass in the ECC thrombochamber. **B)** Surface area of thrombus formation in the ECC thrombochamber, which was quantified by software imaging. Data represents mean ± standard deviation. n=6 for NO, LI, & LINOREL. For SR, n=6 for 1 h, n=5 for 2 h, n=4 for 3 h and 4 h. Statistical significance is indicated by * ($p \leq 0.05$).

	Sample	Baseline	1 h	2 h	3 h	4 h
Flow Rate	Control	60.66 ± 21.16	52.2 ± 16.93	57.5 ± 17.94	75.75 ± 18.10	68.00 ± 31.29
	NO	66.33 ± 10.60	89.5 ± 10.41	100.67 ± 15.33	104.50 ± 13.67	117.50 ± 24.35
	LI	79.83 ± 25.69	87.00 ± 27.11	105.00 ± 25.77	112.33 ± 30.49	122.33 ± 32.07
	LINO	74.00 ± 23.13	93.83 ± 22.83	106.00 ± 11.87	109.50 ± 23.47	103.00 ± 24.50
MAP	Control	45.33 ± 12.13	44.40 ± 15.57	49.75 ± 30.36	53.25 ± 22.79	53.00 ± 26.05
	NO	47.83 ± 4.49	46.17 ± 5.53	49.17 ± 6.11	50.33 ± 5.92	55.17 ± 8.56
	LI	45.33 ± 14.77	45.00 ± 15.90	47.17 ± 17.80	47.00 ± 11.47	50.67 ± 16.52
	LINO	52.17 ± 15.46	50.5 ± 8.34	53.33 ± 8.01	59.00 ± 7.69	60.00 ± 11.61
Heart Rate	Control	180.83 ± 22.22	184.6 ± 28.32	176.50 ± 32.36	184.00 ± 31.23	190.25 ± 31.47
	NO	203.83 ± 25.20	194.5 ± 11.81	199.50 ± 24.93	198.50 ± 26.88	212.33 ± 25.82
	LI	193.83 ± 21.00	191.17 ± 8.68	204.00 ± 14.44	207.33 ± 14.02	211.33 ± 23.83
	LINO	209.17 ± 26.79	196.67 ± 31.10	205.83 ± 23.28	219.00 ± 21.52	216.00 ± 28.57

Table 1: ECC Flow Rate and rabbit vitals (mean arterial pressure [MAP] and Heart Rate) over time. n=6 for NO, LI, & LINOREL. For SR, n=6 for 1 h, n=5 for 2 h, n=4 for 3 h and 4 h

b) Conclusions/Achievements: UGA completed the rabbit testing (n=6/group), as interim data analysis indicated significant differences were observed and additional studies not required. Compared to silicon control, the LiNORel tubing significantly reduced adherent thrombus mass/surface area, and also reduced platelet consumption at 3- and 6-hours circulation time. All modified tubings remained patent for 4 hours, while 2 control circuits occluded early. Final data analysis and start of manuscript preparation is now ongoing.

IV. Subtask 4: In vitro evaluation of the liquid-infused/NO polymer coating in full-scale ECLS ex vivo set-up with porcine donor blood.

A. Objective 4a: Write laboratory protocol, receive regulatory approval
   a) Results/Findings/Developments: Reported/completed in Y1
   b) Conclusions/Achievements: Reported/Completed in Y1

B. Objective 4b-e: Write laboratory protocol, receive regulatory approval
   a) Results/Findings/Developments: UGA has prepared and shipped all 10 full-scale ECLS tubing segments to San Antonio for testing with the Geneva Team. To date, the Geneva Team has completed 9 of 10 circulation studies in which tubing from 4 groups (Control silicone, Silicone Oil only, NO Release only, LiNORel) are tested simultaneously with blood from 1 swine donor. A detailed interim analysis was performed and reported in the Y2Q3 Report when 6 of 10 circulation studies had been completed. At the interim analysis, the LiNORel tubing showed significantly better preservation of platelet count compared to Control silicone. Further, the control group required numerically greater heparin administration to maintain the target ACT of 125-160 seconds, compared to all the modified tubing groups. In the next reporting period, the Geneva team will complete the final 6-hour circulation study and perform the complete data set analysis. These results will be reported in the next quarterly report.

   Following each 6-hour ex vivo circulation study, the Geneva team sent tubing from distinct sections (pre-blood pump, post-blood pump, pre-blood reservoir entry point, post-blood reservoir exit point) in the ex vivo circulation test loops to UGA to assess post blood exposure nitric oxide release. **Figure 5** contains the post-flow analysis performed at UGA, which shows expected

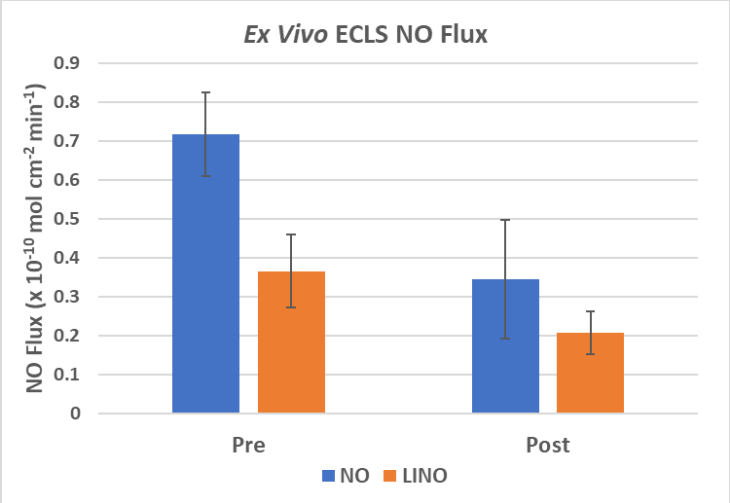

Figure 5: Preliminary Ex Vivo ECLS NO Flux data. Results shown are mean ± std (n=8 for Pre-Exposure, n=6 for Post-Exposure).

results based on their previous publications. The LiNORel loops have a slightly lower NO release compared to the NO only tubing, which is due to the silicone oil preventing stimuli from causing nitroso group release. All NO releases are lower after the ex vivo experiment, which is typical of any NO-releasing material. Current data is showing no significant difference between where tubing segments are located along the ECLS circuit respective to the blood pump and reservoir.

b) Conclusions/Achievements: The 6-hour ex vivo circulation study evaluating full-scale ECLS circuit tubing at clinically relevant flow rates (1.5 Lpm) is nearly complete (n=9 of 10). Results will be finalized, analyzed and reported in the next periodic report.

Major Activity: Determine device performance and systemic effects of LINOREL-coated ECLS circuitry in 72-hour in vivo experiment in healthy swine.

V. *Subtask 5: In vivo evaluation of the LiNORel coating in **uninjured** swine.*

A. Objective 1a: Write animal use protocol, receive regulatory approval

a) Results/Findings/Developments: ACURO approved the animal use protocol on 08 April 2021. All animal protocol approvals acquired.

b) Conclusions/Achievements: Completed on 08 April 2021

VI. *Subtask 6: In vivo evaluation of the LiNORel coating in **injured** swine.*

A. Objective 1a: Write animal use protocol, receive regulatory approval

a) Results/Findings/Developments: ACURO approved the animal use protocol on 08 April 2021. All animal protocol approvals acquired.

b) Conclusions/Achievements: Completed on 08 April 2021

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

Geneva team laboratory research technicians, who aspire to become physicians, received significant professional development opportunities in contributing to animal protocol preparation and in training to use the coagulation and clinical laboratory instruments that are involved in this project. Further, the graduate students in Dr. Handa's lab are training to become biomedical engineers and have the opportunity through this project to be involved in a clinically relevant medical device development project. For example, Mr. Ryan Devine helped with rabbit studies and surgeries, and Manjyot Chug worked with blood and was trained to do blood analysis. Additionally, Dr. Roberts from Dr. Batchinsky's team was invited to give presentations summarizing the translation biomaterial research efforts involved in this project at the 35th Annual Meeting of the Japanese Association for the Surgery

of Trauma; the 37th Annual Advances in Care Conference-Advances in Therapeutics and Technology: Critical Care of Neonates, Children and Adults; and the 2021 ASAIO Conference.

How were the results disseminated to communities of interest?

The Geneva and UGA team published a joint article detailing approaches and testing of biomaterials for ECLS that was published in the Journal of Trauma and Acute Care Surgery Military Health Supplement. The team also drafted another review paper covering methods of blood compatibility testing and clinical relevance of those methods which was submitted to ACS Biomaterials Science & Engineering Journal and is under review. We were unable to attend conferences to present these results this year, as conferences were cancelled due to COVID-19; however, Dr. Roberts from the Geneva team presented at 4 virtual conferences to give invited talks on biomaterial development and testing efforts for ECLS. Both the UGA and Geneva team plan to present the rabbit and ex vivo material testing results reported here as conferences resume in the next year.

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

In the next reporting period, the ex vivo circulation testing of LiNORel tubing will be completed. Post-study sample processing and analysis will be performed. Both groups will begin drafting manuscripts to summarize the material characterization results, the *in vivo* rabbit testing results, and the *ex vivo* full-scale ECLS circulation model. The Geneva team will train personnel and plan for the 72-hour *in vivo* large animal testing phase of the study. This will include familiarizing the team with use of the NO Gas generator that will provide NO gas into the membrane oxygenator during ECLS in the LiNORel test group. The UGA team will prepare to make ECLS tubing circuits for the animal study.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Through this collaborative effort, we investigated a novel method for application of NO donor species to ECLS membrane oxygenator fibers using a solvent-swelling method. This is a novel coating application method for this specific application which we intend to detail in a joint manuscript. The efforts to apply the material modifications to ECLS-specific devices and surfaces can inform other biomaterial efforts.

This project has also led the Geneva team to develop contacts at Maquet/Getinge Group (ECLS device manufacturer/distributor) and Membrana (ECLS membrane oxygenator fiber manufacturers). We have successfully established points of contact with these groups and maintain regular meetings with them to discuss their vision of a successful ECLS biomaterial. The Geneva team is able to share our experience gained through the project to work with these groups to identify important blood screening tests and models, and to identify key challenges that clinician scientists and bioengineers have identified through this project that the device manufacturers are not necessarily aware of.

What was the impact on other disciplines?

Development of a solution to minimize coagulation disturbance during ECLS will revolutionize medical perception of ECLS and will enable use of ECLS in traumatically wounded with hemorrhagic conditions. Further, this project is exceptionally multidisciplinary where researchers with backgrounds in chemistry, biology, physiology, engineering and/or veterinary medicine and clinical medicine are working together. This is an invaluable experience for students to learn in a fast pace multidisciplinary research environment and learn to communicate with each other where the backgrounds are very different. We believe this research will impact medicine, chemistry, medical device industry, veterans, and current military personnel.

What was the impact on technology transfer?

Nothing to report at this stage.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

We identified a problem with loading of SNAP donor into the membrane oxygenators – as the high surface area/low volume of the fibers leads to rapid NO offloading with no residual NO reserve for sustained, multi-day NO release. As proposed and approved following the Y1 annual report, we plan to use NO gas administered into oxygenator sweep gas, rather than applying LiNORel to the membrane oxygenators, enabling us to proceed to in vivo testing.

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

a. Actual Problems or delays and actions to resolve them

A shortage of plastics related to COVID-19 supply chain disturbances caused a delay in acquiring tubings for the full-scale ex vivo circulation study; however, tubing was received in sufficient quantities to finish the ex vivo testing and begin preparations for animal testing. Covid-19 related laboratory closures at UGA early in 2021 delayed the rabbit studies and ex vivo circulation studies; however, actions were taken such that these studies are now nearly completed. These delays have pushed back the start of the large animal experiments, but everything is in place to start large animal testing as we complete the rabbit and ex vivo studies.

b. Anticipated Problems/Issues

N/A

Changes that had a significant impact on expenditures

N/A

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

N/A

Significant changes in use or care of human subjects

N/A – no human subjects

Significant changes in use or care of vertebrate animals

None.

Significant changes in use of biohazards and/or select agents

N/A – no use of biohazards and/or select agents

TOTAL PROTOCOL(S): 3 animal use research protocols will be required to complete the Statement of Work.

PROTOCOL (_1 of 3_ total):
Protocol [ACURO Assigned Number]: JW180016.e002
Title: Combating thrombosis and infection by NO releasing/generating polymers
Target required for statistical significance: 40
Target approved for statistical significance: 40

SUBMITTED TO AND APPROVED BY:
University of Georgia IACUC approved original protocol (protocol number A2018 12-005, PI Dr. Hitesh Handa) on 18 Jan 2018. ACURO approved the protocol (JW180016.e001) on 28 September 20. This protocol expired on 18 January 2021 (at which time all animal work ceased). A new protocol was drafted and submitted to the University of Georgia IACUC and approved on 24 Dec 20 (protocol number A2020 12-003). This new protocol was approved by ACURO (JW180016.e002) on 02 April 2021.

STATUS:
UGA IACUC approval 24 Dec 20 (A2020 12-003)
ACURO approval 02 Apr 21 (JW180016.e002)
Animal studies to resume in May 2021.

PROTOCOL (_2 of 3_ total):
Protocol [ACURO Assigned Number]: JW180016.e003
Title: Heparin Free Extracorporeal Life Support for Point of Need Treatment of Single and Multiorgan Failure
Target required for statistical significance: 36
Target approved for statistical significance: 36

SUBMITTED TO AND APPROVED BY:
UTSA IACUC (SU004-02-24) approved on 15 March 21
ACURO (protocol JW10016.e003) approved on 08 April 21

STATUS:
All approvals received; animal studies not yet started.

PROTOCOL (_3 of 3_ total):
Protocol [ACURO Assigned Number]: N/A
Title: GLP Assessment of LiNORel Coating *In Vivo* for 72 Hours Heparin-Free ECLS
Target required for statistical significance: 12
Target approved for statistical significance: N/A

SUBMITTED TO AND APPROVED BY: N/A

STATUS:
Protocol not yet drafted/submitted. On track to be drafted/submitted/approved by month 34. Discussion with GLP facility initiated.

6. PRODUCTS:

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

Journal publications.

Manuscript – Review Article:
Roberts TR, Garren MRS, Handa H, Batchinsky AI. Toward an artificial endothelium: Development of blood-compatible surfaces for extracorporeal life support. *J Trauma Acute Care Surg.* 2020; 89(2S Suppl 2): S59-S68.

Manuscript – Review Article:
Devine R, Ashcraft M, Roberts TR, Batchinsky AI, Handa H. Medical device hemocompatibility: Mechanisms, testing, methods, and clinical translation. *Submitted to ACS Biomaterials Science and Engineer* (Under review).

Books or other non-periodical, one-time publications. None

Other publications, conference papers and presentations.

Roberts, TR. Bio-inspired surfaces and regional anticoagulation strategies for extracorporeal life support: material assessment and development protocol for clinical translation. *35th Annual Meeting of Japanese Association for the Surgery of Trauma.* Tokyo, Japan. 28 May 2021 (invited lecture, virtual).

Roberts, TR. Coagulation management approaches for extracorporeal organ support in the pre-hospital setting. *Brooke Army Medical Center – Burn Center Invited Clinical Science Lecture.* San Antonio, TX, USA. 07 July 2021 (invited lecture).

Roberts, TR. Innovation in antithrombogenic coatings for extracorporeal life support. *37th Annual Advances in Care Conference – Advances in Therapeutics and Technology: Critical Care of Neonates, Children and Adults.* Snowbird, Utah, USA. 24-26 March 2021 (invited lecture, virtual).

Roberts, TR; Harea, GT; Batchinsky, AI. Relevance of ex vivio blood circulation models to in vitro extracorporeal circulation on coagulation outcomes. *66th Annual ASAIO Conference.* Chicago, IL, USA. 8-11 June 2021 (oral presentation, virtual).

Roberts, TR, Batchinsky, AI. Coagulation management approaches for extracorporeal pulmonary support in the pre-hospital setting. *66th Annual ASAIO Conference.* Chicago, IL, USA. 8-11 June 2021 (invited lecture, virtual).

- **Website(s) or other Internet site(s)**
  None

- **Technologies or techniques**
  None

- **Inventions, patent applications, and/or licenses**
  None

- **Other Products**
  None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Andriy Batchinsky, MD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	ORCID 0000-0001-8601-2827

Nearest person month worked: 1.0
Contribution to Project: Overseeing conduct of the study, supervised study execution, data and sample collection and analysis, coordinating preparation of manuscripts and reports.

Name: Teryn Roberts, PhD
Project Role: Co-PI
Researcher Identifier (e.g. ORCID ID): ORCID 0000-0002-2460-6432
Nearest person month worked: 2.1
Contribution to Project: Overseeing conduct of the study, supervising study execution, data and sample collection and analysis, experiment planning, report and manuscript preparations.

Name: Jae Choi, PhD
Project Role: Co-PI
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.5
Contribution to Project: Overseeing conduct of the study, supervising study execution, data and sample collection and analysis, and coordinating the preparation of manuscripts and reports.

Name: Hitesh Handa
Project Role: Co-PI
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.0 mo
Contribution to Project: Dr. Handa participated in meetings with researchers from his lab and Dr. Batchinsky's group. He is involved in planning and execution of the project. He is guiding students and leading the effort to develop the hemocompatible/antibacterial surfaces.

Name: Ryan Devine
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12.0 mo
Contribution to Project: Developing NO releasing and silicone oil swelled tubings.

Name: Rashmi Pandey
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 6.0 mo
Contribution to Project: Rabbit studies and blood sample analysis

Name: Anil Kumar
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID): ORCID N/A
Nearest person month worked: 6.0 mo
Contribution to Project: Dr. Kumar synthesized SNAP (NO donor) and researched and developed methods to swell membrane fibers with SNAP. He is working to fabricate NO releasing tubing swelled with silicone oil.

Name: Blake Shessel
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID): ORCID N/A
Nearest person month worked: 5 months

Contribution to Project: Blake is a veterinarian and she planned and conducted all the rabbit surgeries.

Name: Manjyot Kaur
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): ORCID N/A
Nearest person month worked: 6 months
Contribution to Project: Manjyot helped with testing blood samples drawn every hour from rabbit such as aggregometer studies etc.

Name: Lori Estes
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): ORCID N/A
Nearest person month worked: 2.0 mo
Contribution to Project: Conduct bacteria studies.

Name: Arnab Mondal
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 6.0 mo
Contribution to Project: Conduct bacteria studies.

Name: Brendan Beely
Project Role: Research Coordinator
Researcher Identifier (e.g. ORCID ID): 0000-001-9442-9462
Nearest person month worked: 1.8
Contribution to Project: Assisting with study protocols and report preparation, routine laboratory procedures.

Name: Dan Wendorff
Project Role: Laboratory Manager
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.7
Contribution to Project: Animal protocol preparation, oversee lab technicians, routine laboratory procedures.

Name: Hailee Alaniz
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.5
Contribution to Project: Assisting with large animal protocol preparation and handling

Name: Isabella Garcia
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.7
Contribution to Project: Assisting with large animal handling and protocol preparation

Name: Clayton Smith
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.3
Contribution to Project: Assisting with large animal handling and protocol preparation

Name: Robert Willis

Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
earest person month worked: 0.4
Contribution to Project: Protocol preparation, assisting with data collection and interpretation.

Name: Ji Lee
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.6
Contribution to Project: Assisting with data collection and interpretation.

Name: Adriana Fuentealba
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.4
Contribution to Project: Assisting with data collection and interpretation.

Name: Zachary Allen
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.2
Contribution to Project: Assisting with data collection and interpretation.

Name: Yanyi Zang
Project Role: Postodctoral Fellow
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.3
Contribution to Project: Assisting with ex vivo circulation study execution and post-circulation material analysis, biosample processing

Name: Brittney Lewis
Project Role: Regulatory Compliance Specialist
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.8
Contribution to Project: Protocol drafting and preparations.

Name: George Harea
Project Role: Research Associate II
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.0
Contribution to Project: Assisting with ex vivo circulation study execution and post-circulation material analysis, biosample processin

Name: John Jones
Project Role: Statistician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.2
Contribution to Project: Data analysis and interpretation.

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

None.

What other organizations were involved as partners?

Organization Name: University of Georgia Research Foundation, Inc.
Location of Organization: 343, Tucker Hall, 310 E Campus Rd, Athens, GA 30602
Partner's contribution to the project (identify one or more): Collaboration

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS: Attached.