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

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13. SUPPLEMENTARY NOTES

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 lifesaving 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 lifesaving 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	100%
Objective 3c: Experimental group 2: NO release only **(n=10)**	11-18		Dr. Handa	100%
Objective 3d: Experimental group 3: Liquid infused only **(n=10)**	11-18		Dr. Handa	100%

Objective 3e: Experimental group 4: Candidate LINOREL **(n=10)**	11-18		Dr. Handa	100%
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	100%
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		100%
Objective 4c: Experimental group 2: NO release only **(n=10)**	11-18	Dr. Batchinsky		100%
Objective 4d: Experimental group 3: Liquid infused only **(n=10)**	11-18	Dr. Batchinsky		100%
Objective 4e: Experimental group 4: Candidate LINOREL **(n=10)**	11-18	Dr. Batchinsky		100%
MILESTONE: Complete ex vivo ECLS model	11-18	Dr. Batchinsky	Dr. Handa	100%
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	10%
Objective 5c: Experimental group 2: ECLS with LINOREL combination coating without systemic heparinization **(n=9)**	18-24	Dr. Batchinsky	Dr. Handa	20%
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	10%
MILESTONE: Complete post-explantation SEM analysis of membranes after *in vivo* use	36	Dr. Batchinsky	Dr. Handa	10%
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.

II. *Subtask 2: Assess the bacteriostatic and antibacterial properties of LiNORel.*
-Subtask 2 completed in Y2, please see Y2 Annual Report for findings and conclusions.

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 4 hour rabbit extracorporeal circulation study has been completed during the Y3 reporting period. The study supports the notion that LiNORel tubing modification improves tubing hemocompatibility *in vivo* relative to unmodified control. Figure 1 shows thrombus mass/surface area coverage on the tubing at the end of the experiment; as well as the percent patency of the circuits throughout the study course (Figure 1C). Figure 2 shows the results of the platelet count, fibrinogen concentration and white blood cell count throughout circulation. Figure 3 shows expression of platelet P-selectin and GP IIb/IIIa with and without collagen stimulation; as well as platelet activation. Table 1 shows the vital signs and circuit blood flow conditions.

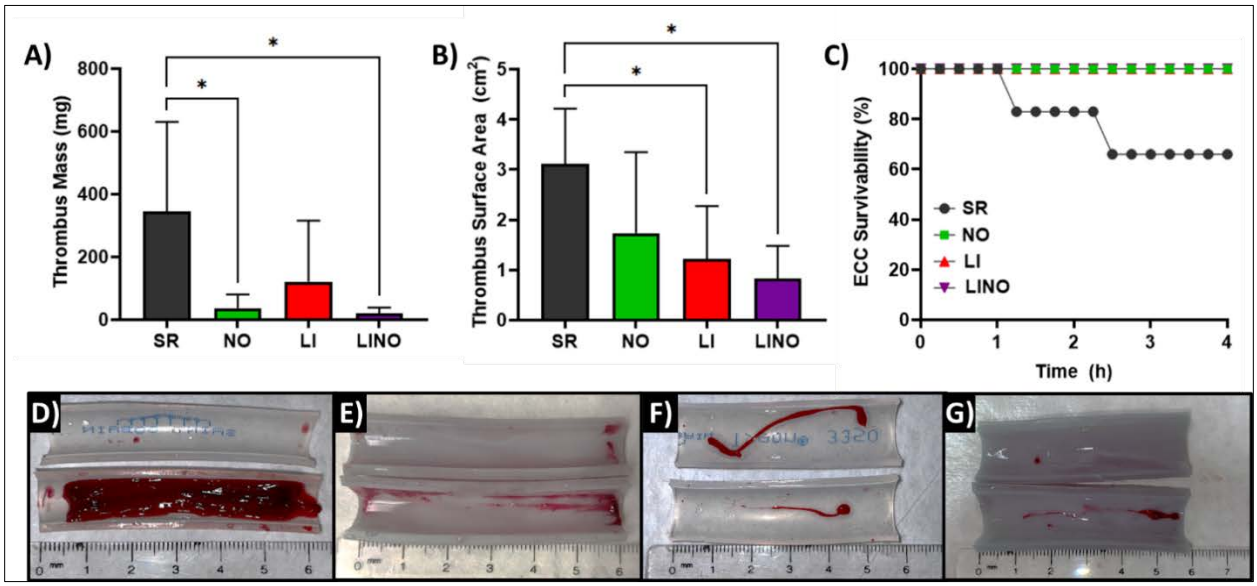


Figure 1- A) Thrombus mass in the ECC thrombochamber. **B)** Surface area of thrombus formation in the ECC thrombochamber, which was quantified by software imaging. **C)** ECC survivability over the 4 h flow study. **D-G)** Thrombochamber images of control SR (D), NO (E), LI (F), LINO (G). Data represents mean ± standard error of mean (n=6). Statistical significance is indicated by * ($p \leq 0.05$).

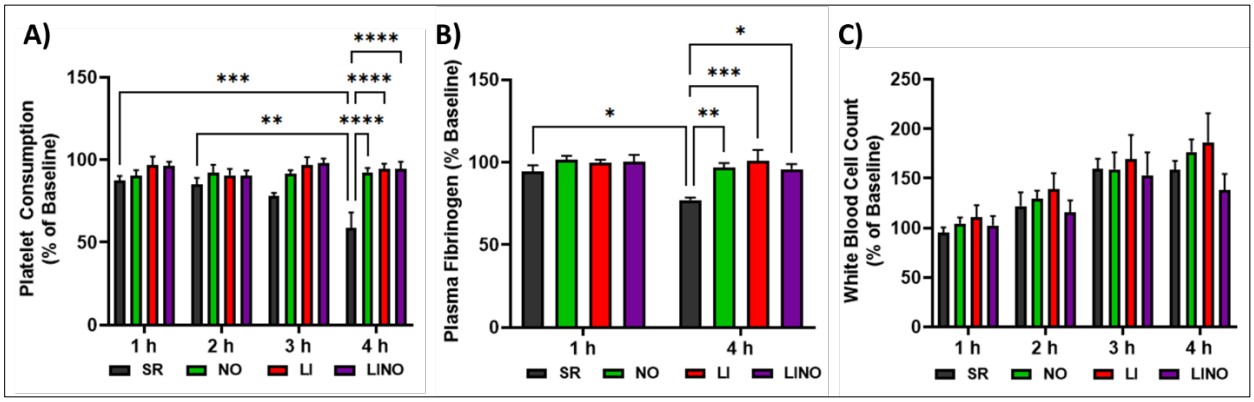


Figure 2: Data represents mean ± standard error of mean. n=6 for NO, LI, & LINO. For SR, n=6 for 1 h, n=5 for 2 h, n=4 for 3 h and 4 h. Statistical significance is indicated by * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

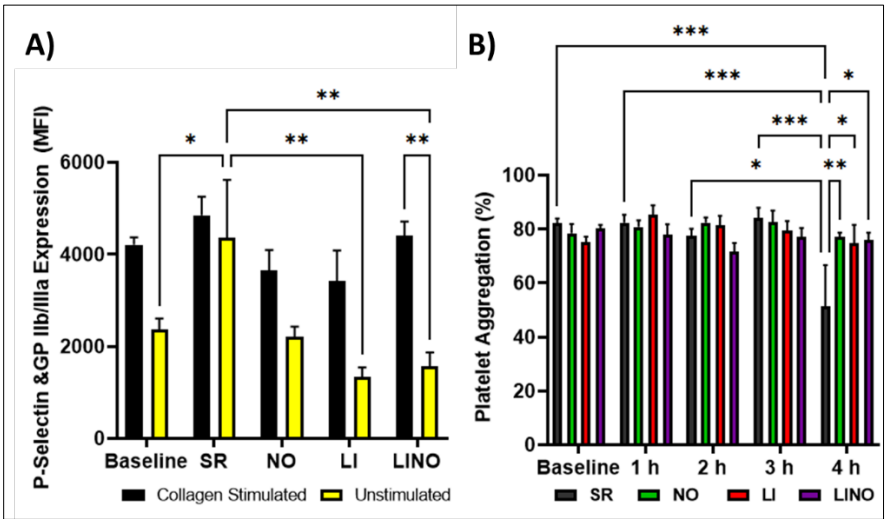


Figure 3: Platelet **A)** Expression of P-Selectin & GP IIb/IIIa on platelet cells with and without collagen stimulation after 4 h of flow (n=3). **B)** Surface area of thrombus formation in the ECC thrombochamber, which was quantified by software imaging (n=6 for NO, LI, & LINO. For SR, n=6 for 1 h, n=5 for 2 h, n=4 for 3 h and 4 h). Data represents mean ± standard error of mean. Statistical significance is indicated by * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

	Sample	Baseline	1 h	2 h	3 h	4 h
Flow Rate (mL min^{-1})	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, rabbit vitals (mean arterial pressure [MAP] and Heart Rate). n=6 for NO, LI, & LINO. For SR, n=6 for 1 h, n=5 for 2 h, n=4 for 3 h and 4 h. Statistical significance compared to control is indicated by * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

b) Conclusions/Achievements: UGA has completed the rabbit study and the manuscript summarizing this work is in final stages of preparation prior to submission for peer review and publication. Results suggest that LiNORel improves hemocompatibility in a low flow *in vivo* setting without eliciting untoward effects.

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: The AREVA/Geneva team completed the *ex vivo* full-scale ECLS circuit tubing experiment in the Y3 reporting period. Additionally, UGA completed the post-circulation analysis of the tubing within this reporting period. Results as follows in Figures 4-6 and Table 2:

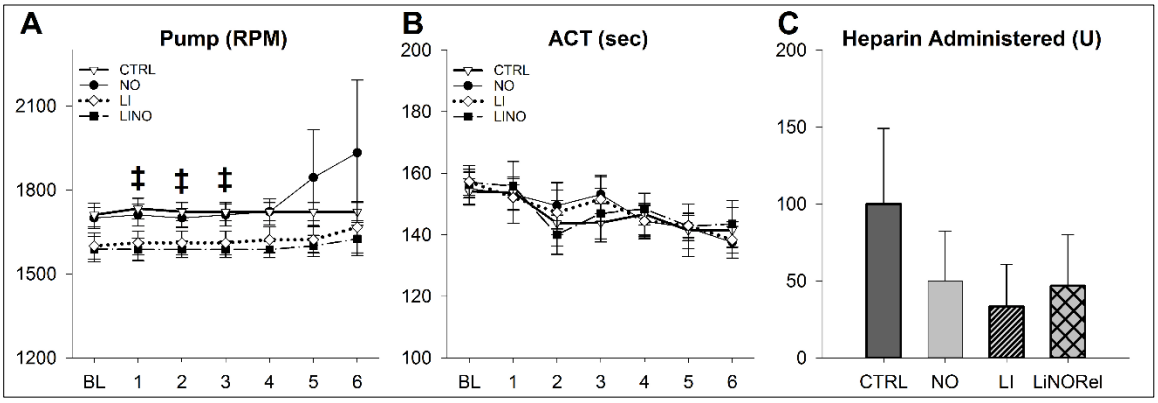


Figure 4: A) Blood pump revolutions per minute (RPM) to achieve target flow rate of 1.5 L/min; **B)** activated clotting time (ACT); and **C)** total quantity of unfractionated heparin administered to maintain ACT between 125-160 s during 6 h hemocompatibility testing of extracorporeal circulation tubing using swine blood. Standard medical grade silicon rubber tubing (CTRL) was compared to nitric oxide-releasing tubing (NO), non-adhesive liquid infused tubing (LI), and dual-action [illegible] between CTRL and [illegible] $p<0.05$ for signific[illegible]

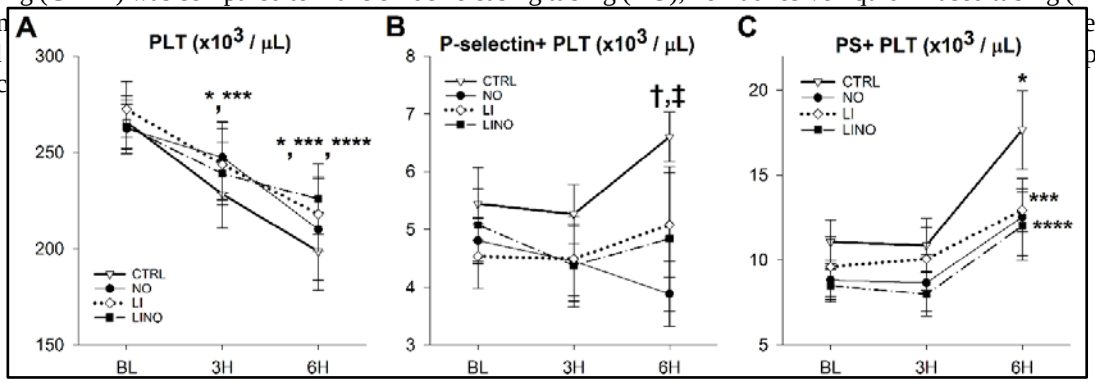


Figure 5: A) Platelet (PLT) count; **B)** concentration of activated platelets indicated by P-selectin expression; and **C)** procoagulant platelets indicated by phosphatidyl serine (PS) expression during 6 h hemocompatibility testing of extracorporeal circulation tubing using swine blood. Standard medical grade silicon rubber tubing (CTRL) was compared to nitric oxide-releasing tubing (NO), non-adhesive liquid infused tubing (LI), and dual-action nitric oxide release liquid infused tubing (LINO), n=9/group. Significant changes within groups over time compared to baseline (BL) for *****CTRL, ******NO, *******LI, and ********LINO are indicated. †Indicates significant difference between CTRL and NO groups. ‡Indicates significant difference between CTRL and LINO groups. All tests were two-sided with $p<0.05$ for significance.

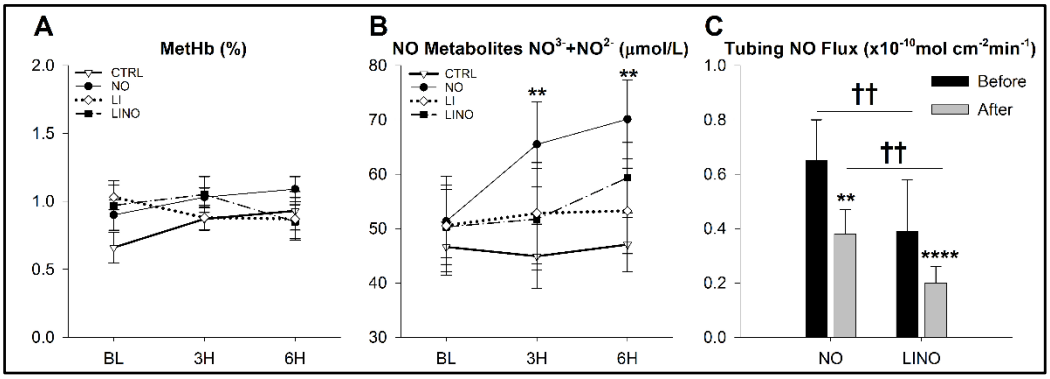


Figure 6: A) Methemoglobin fraction (MetHb); **B)** plasma concentration of nitric oxide (NO) metabolites nitrate and nitrite during 6 h hemocompatibility testing of extracorporeal circulation tubing using swine blood. Standard medical grade silicon rubber tubing (CTRL) was compared to nitric oxide-releasing tubing (NO), non-adhesive liquid infused tubing (LI), and dual-action nitric oxide release liquid infused tubing (LINO), n=9. **C)** Flux of nitric oxide from the NO and LINO tubing surfaces before and after 6 h blood circulation (Before, n=9; After, n=54). Significant changes within groups over time compared to baseline (BL) for *****CTRL, ******NO, *******LI, and ********LINO are indicated. †† Indicates significant differences between NO and LINO groups. All tests were two-sided with $p<0.05$ for significance.

	NO	LINORel
Pre-Exposure NO Flux	0.65 ± 0.15	0.38 ± 0.09
Post-Exposure NO Flux	0.39 ± 0.19	0.20 ± .06
A-Bag NO Flux	0.45 ± 0.17	0.20 ± 0.06
A-Mid NO Flux	0.46 ± 0.26	0.18 ± 0.08
A-Pump NO Flux	0.41± 0.23	0.21 ± .07
B-Bag NO Flux	0.35 ± 0.15	0.24 ± .08
B-Mid NO Flux	0.32 ± .12	0.18 ± 0.05
B-Pump NO Flux	0.36 ± 0.20	0.18 ± 0.05

Table 2: NO Flux pre and post exposure to blood. Data represented is in units of (x 10^{-10} mol cm^{-2} min^{-1}) and is represented as mean ± std. n=9 for all sample types except for Post-Exposure NO Flux, which is n=54.

b) Conclusions/Achievements: The *ex vivo* study demonstrated that using flow conditions that are clinically utilized for partial lung support, the LiNORel coating does not elicit any untoward effects on blood. For example, NO flux did not cause elevated methemoglobin fraction or excessive accumulation of NO metabolites. Additionally, we observed a significant lower level of activated (P-selectin expressing) platelets at 6 h circulation time in the NO and LiNORel modified tubing loops versus control. Further, this study demonstrated that LiNORel can be scaled for application to full-size ECLS devices. Further, we demonstrated that LiNORel tubing continues to release NO following 6 h blood exposure. This work has been drafted into a manuscript and is currently under peer review for consideration for publication.

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 5a: Write animal use protocol, receive regulatory approval
   a) Results/Findings/Developments: Completed in Y2.
   b) Conclusions/Achievements: Completed in Y2.

B. Objective 5b-c: Experimental group 1 -- ECLS with manufacturer's standard circuit (with immobilized heparin coating) and continuous heparinization (n=9) and Experimental group 2 – ECLS with LiNORel combination coating + NO gas applied to membrane oxygenator without systemic heparinization (n=9).
   c) Results/Findings/Developments: In Year 3, the AREVA/Geneva team has completed n=2 of the experimental group studies. No obvious untoward effects were noted. Both circuits remained patent for the 72-hour study duration tested. An interim data analysis will be performed once additional control group and experimental group studies are completed. After each study, tubing samples were rapidly prepared and shipped to UGA for post-circulation analysis of coating stability.
   d) Conclusions/Achievements: 72-hour animal studies have been initiated, and to date the LiNORel approach has not caused obvious untoward effects and enables 72-hours ECLS without systemic anticoagulation. Further studies will continue in the next reporting period.

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: Completed in Y2
   b) Conclusions/Achievements: Completed in Y2

VII. Subtask 7: Assess coating stability after 72 hours in vivo testing.

A. Objective 7a: Circuit components and tubing samples will be analyzed by the AREVA team and UGA team following 72 hours us in animal studies.
   a) Results/Findings/Developments: Following the n=2 72-hour animal experiments, the LiNORel ECLS circuits were carefully explanted, rinsed and imaged/dissected to quantify and characterize the degree of thrombus deposition on the circuit surfaces at the end of each multi-day study. At AREVA/Geneva, tubing samples are collected from the pre-membrane/venous blood circulation line; as well as from the post-membrane/arterial blood circulation line. Additionally, samples of the dual-lumen ECLS cannula which is not LiNORel coated is collected and dissected for SEM imaging. Samples are dehydrated at AREVA/Geneva, and then shipped to UGA for SEM imaging, as well as for measurement of post-circulation NO flux and sliding angle from the LiNORel tubing. At AREVA/Geneva, the membranes are dissected and digital images collected of all membrane layers to determine the total area thrombus deposition coverage in the gas exchange fiber stack.

Table 3 shows the NO flux from the 2 LiNORel circuits tested – pre-blood exposure measurements are compared to post-72 hours in vivo application. Likewise, sliding angle which is an assessment of the slippery characteristic of the LiNORel coating was compared before and after tubing use in vivo (Table 4):

	LINORel
Pre-Exposure NO Flux	0.16 ± 0.03
Post-Exposure NO Flux	0.47 ± 0.15
Pre-Center NO Flux	0.40 ± 0.09
Post-Center NO Flux	0.38 ± 0.23
Pre-Patient End NO Flux	0.67± 0.18
Post-Patient End NO Flux	0.47 ± 0.10
Pre-Pump End NO Flux	0.44 ± 0.04
Post-Pump End NO Flux	0.48 ± 0.22
Excess Tubing (No Blood) NO Flux	0.33 ± 0.09

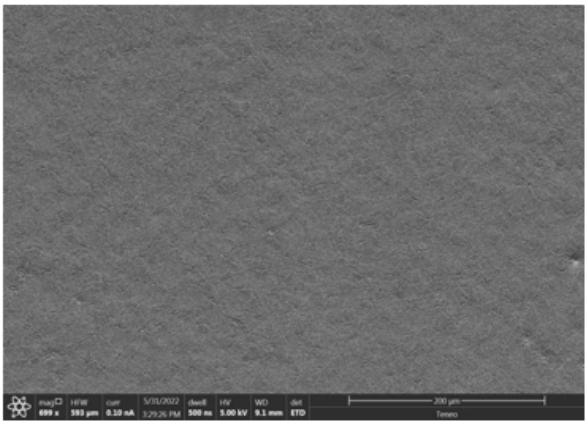
Table 3. NO flux for pre and post exposure to blood. Post-exposure is broken down into 6 sections to represent different locations of the loop as described by their name. Data represented is in units of (x 10^{-10} mol cm^{-2} min^{-1}) and as mean ± standard deviation. n=2 for all sample types, except Post-exposure NO flux, which is n=12.

	Sliding Angle (°)
Pre-Exposure Sliding Angle	24.62
Post-Exposure Sliding Angle	34.34 ± 10.69
Pre-Center Sliding Angle	32.28
Post-Center Sliding Angle	25.61
Pre-Patient End Sliding Angle	53.72
Post-Patient End Sliding Angle	39.00
Pre-Pump End Sliding Angle	25.95
Post-Pump End Sliding Angle	29.49

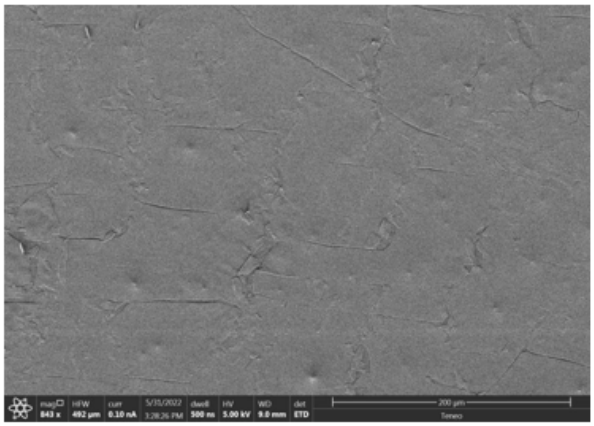
Table 4. Sliding angle for pre and post exposure to blood. Post-exposure is broken down into 6 sections to represent different locations of the loop as described by their name. Data shown is n=1, except for Post-Exposure Sliding Angle which is n=6 and shown as mean ± standard deviation, data is from the loop PM001. *Note: Loop PM002 is not shown in this table due to all angles being >90°* indicating no slippery behavior.

Representative SEM images of LiNORel coated tubing (Figure 7) and uncoated catheter (Figure 8) from one animal are shown below:

A)



B)



C)

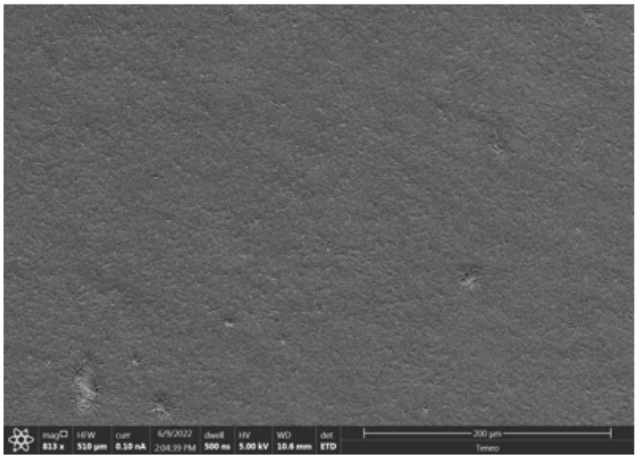


Figure 7. SEM images taken from various locations of LINORel modified tubing used in pig study ULIN02. Images shown are representing the locations of A) Pre B) Post and C) extra tubing from ULIN02. The same naming convention from ULIN01 (Pre, Post, extra tubing) applies here for the images from ULIN02. All tubing shown in this figure were LINORel modified.

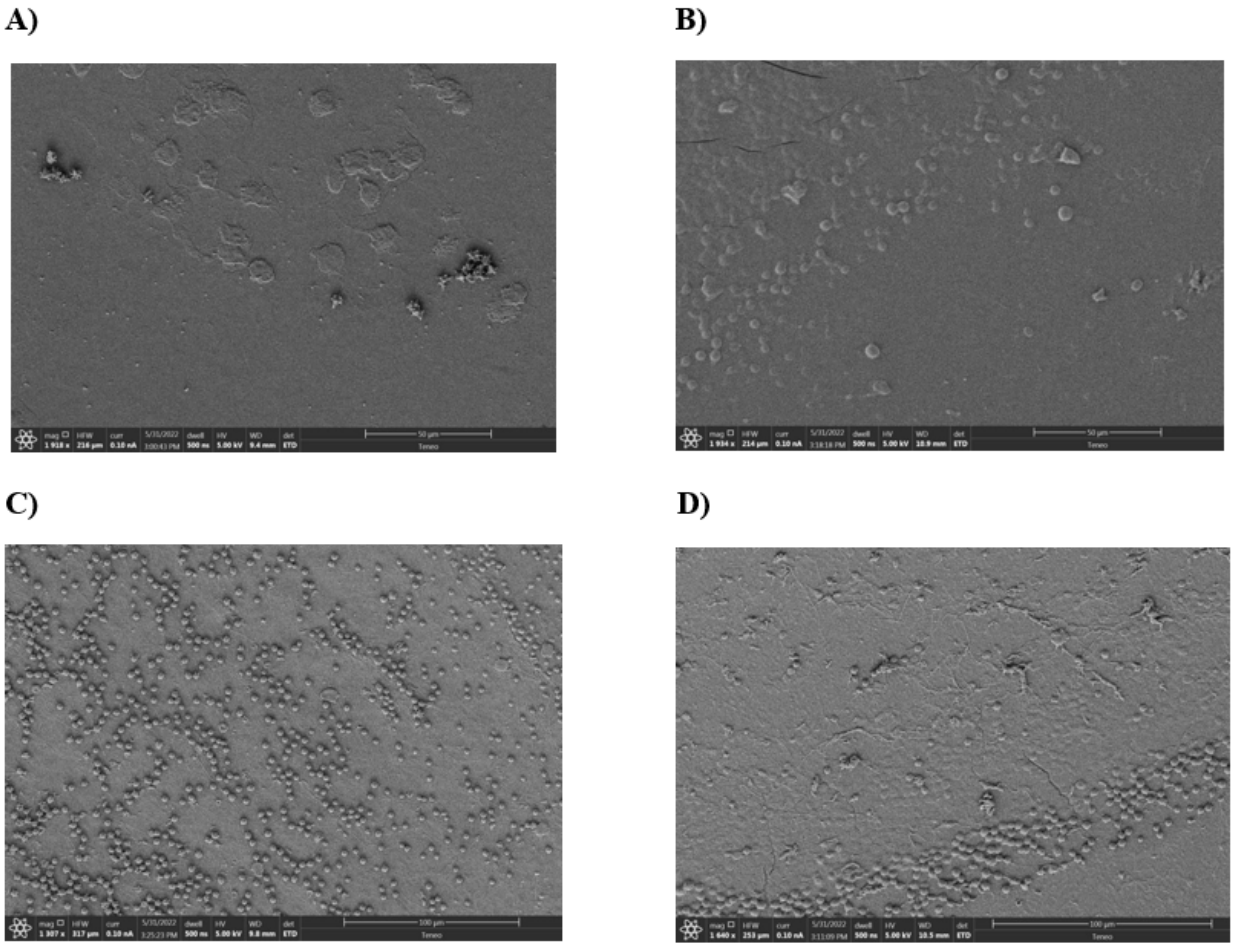


Figure 8. SEM images from sections of unmodified medical grade catheter used in the pig study ULIN02 separated into sections based on location of the catheter A) catheter inlet B) catheter middle C) catheter outlet and D) catheter tip. All sections of the catheter were not modified at all.

b) Conclusions/Achievements: Post-circulation analysis of materials following 72 h animal studies is underway and will continue in the next reporting period.

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

At the AREVA/Geneva 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 at UGA 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 successfully defended his PhD dissertation within this reporting period. He

has now transitioned to a biomedical research career in industry at StimLabs (Roswell, GA). Other students learned to perform *in vitro* blood testing methods. Additionally, Mr. George Harea who is a PhD student in tratining from Dr. Batchinsky's team was invited to give a presentation summarizing this translational biomaterial research effort at the 2022 ASAIO Conference (Chicago, IL; 09 June, 2022). Dr. Roberts and Dr. Batchinsky from AREVA were invited faculty who presented seminars relevant to this work at the 38th Annual Advances in Care Conference: Adavances in Therapeutics and Technology – Critical Care of Neonates, Children, and Adults (Snowbird, UT; March 31, 2022). Ms. Niemeyer, a premedical student research technician at the AREVA/Geneva lab was invited to present portions of this work at the virtual ECMO and the Advanced Therapies for Respiratory Failure Symposium (virtual; February 2022).

How were the results disseminated to communities of interest?

Results were presented at highly relevant clinical and scientific conferences including ASAIO annual conference, CNHS Annual ECMO and Advanced Therapies for Respiratory Failure Symposium, and the Snowbird 38th Advances in Therapeutics and Technology Conference. The ex vivo study has been written into manuscript format and is under review for publication in a biomaterial science journal. The rabbit study is in the final stages of manuscript preparation and will be submitted for publication shortly.

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

In the next reporting period, UGA will continue to make LiNORel tubing circuits for animal studies and will analyze materials from the 72-hour animal studies following use. The AREVA/Geneva team will continue to execute the 72-hour animal studies. AREVA/Geneva will continue to make progress towards establishing GLP capability within the laboratory. We have designated an individual for oversight and have begun establishing document storage and access procedures to support GLP work.

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 AREVA/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

Nothing to report.

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

a. Actual Problems or delays and actions to resolve them

Nothing to Report.

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 completed.

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 begun (n=2 completed).

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:
Roberts TR, Garren MRS, Wilson SN, Handa H, Batchinsky AI. Development and In Vitro Whole Blood Hemocompatibility Screening of Endothelium-Mimetic Multifunctional Coatings. *ACS Appl Bio Mater*. 2022; 5(5): 2212-2223.

Manuscript:
Roberts TR, Harea GT, Zang Y, Devine RP, Maffe P, Handa H, Batchinsky AI. A dual-action nitric oxide-releasing slippery surface for extracorporeal organ support: First dynamic in vitro hemocompatibility evaluation. *J Appl Biomater Res B (in submission, under review).*

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

Other publications, conference papers and presentations.

Niemeyer C, Zapien R, Warar S, Zang Y, Wick T, Melvin A, Devine RP, Reynolds MM, Handa H, Roberts TR, Batchinsky AI. Comparison of NO metabolite levels during ex vivo extracorporeal circulation of blood using NO-eluting and NO-generating surface coatings. *38th Annual ECMO and Advanced Therapies for Respiratory Failure Symposium.* Keystone, CO, USA. 15 February, 2022 (podium presentation, virtual).

Batchinsky AI, Warar S, Thrailkill M, Quint M, Roberts TR, Wednorff DW, Harea GT, Beely BM. ECLS during COVID: Are we changing practice? *38th Annual Advances in Care Conference – Advances in Therapeutics and Technology: Critical Care of Neonates, Children and Adults.* Snowbird, UT, USA. 31 March, 2022. (invited lecture).

Roberts TR, Batchinsky AI. Anatomy of anticoagulation in blood-polymer interfaces: implications for medical device development. *38th Annual Advances in Care Conference – Advances in Therapeutics and Technology: Critical Care of Neonates, Children and Adults.* Snowbird, UT, USA. 31 March, 2022. (invited lecture).

Devine, RP. Slippery, nitric oxide releasing surfaces for the improvement of medical device hemocompatibility. *Dissertation, Doctor of Philosophy, University of Georgia.* May 2022. (dissertation).

Roberts, TR; Harea, GT; Zang Y, Garcia I, Devine R, Handa H, Batchinsky, AI. A dual-action nitric oxide-releasing slippery surface coating for extracorporeal organ support: first evaluation at clinically relevant blood flow rate for partial lung support . *67th Annual ASAIO Conference.* Chicago, IL, USA. 8-11 June 2022 (podium).

- **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: 2.3
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: 0.1
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: 10 mo
Contribution to Project: Developing NO releasing and silicone oil swelled tubings.

Name: Patrick Maffe
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1 mo
Contribution to Project: Developing NO releasing and silicone oil swelled tubings.

Name: Anil Kumar
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID): ORCID N/A
Nearest person month worked: 1 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: 1 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: 2 months

Contribution to Project:	Manjyot helped with testing blood samples drawn every hour from rabbit such as aggregometer studies etc.
Name:	Brendan Beely
Project Role:	Research Coordinator
Researcher Identifier (e.g. ORCID ID):	0000-001-9442-9462
Nearest person month worked:	2.3
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:	1.0
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:	0.2
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:	1.8
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:	0.2
Contribution to Project:	Protocol preparation, 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:	1.4
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:	4.5
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:	0.2
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: 0.6
Contribution to Project: Assisting with ex vivo circulation study execution and post-circulation material analysis, biosample processin

Name: Cameron Chan
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.1
Contribution to Project: Protocol preparation, assisting with data collection and interpretation.

Name: Kaitelynn Beely
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.1
Contribution to Project: Protocol preparation, assisting with data collection and interpretation.

Name: Shubhneet Warar
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.0
Contribution to Project: Protocol preparation, assisting with data collection and interpretation.

Name: Ryley Zapien
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.0
Contribution to Project: Protocol preparation, assisting with data collection and interpretation.

Name: Cassie Niemeyer
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.0
Contribution to Project: Protocol preparation, assisting with data collection and interpretation.

Name: John Jones
Project Role: Statistician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.5
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.