AWARD NUMBER: W81XWH-18-2-0048

TITLE: Heparin-Free Minimal Invasive Extracorporeal Life Support Treatment of Acute Lung Injury

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CONTRACTING ORGANIZATION: The Geneva Foundation, San Antonio, TX

REPORT DATE: October 2021

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel 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	15Sep2020-14Sep2021

4. TITLE AND SUBTITLE

Heparin-Free Minimal Invasive Extracorporeal Life Support Treatment of Acute Lung Injury

5a. CONTRACT NUMBER
W81XWH-18-2-0048

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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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

The purpose of this project is to develop a blood-compatible surface coating for extracorporeal life support (ECLS) devices to prevent thrombus formation during circulation without use of heparin. The coating catalyzes nitric oxide (NO) release in blood, preventing platelet activation. This would enable ECLS in trauma and combat casualties with coagulopathic complications contraindicating the use of heparin. In this first year of the period of performance we have applied the coating to ECLS tubing and catheters and have demonstrated NO-release and reduction in bacterial adhesion. When compared to clinically available ECLS circuits with anticoagulant coatings (immobilized heparin and albumin) performance of the NO-releasing coating was similar and enabled circulation of donor blood for 6 hours. To date the coating cannot be applied to ECLS membranes due to surface characteristics of the polymer, as discovered by our collaborators at Colorado State. To solve this problem, we propose use of NO gas directly delivered into membrane sweep gas. This alternative strategy is based on some preliminary data from the literature, has been tested in pilot studies by our group and was found to be feasible. Further testing will be performed in vivo in healthy and injured swine carried out to a prolonged field care timeline as envisioned in this grant (72hrs).

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**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	22	**19b. TELEPHONE NUMBER** *(include area code)*

Contents

1. **INTRODUCTION:**

This report serves as an annual report of activities and progress made by Dr. Andriy Batchinsky MD, Principal Investigator at The Geneva Foundation and his team towards completion of work awarded to The Geneva Foundation as part of federal grant W81XWH-18-2-0048, titled "Heparin-Free Minimal Invasive Extracorporeal Life Support Treatment of Acute Lung Injury." The study is performed in collaboration with Dr. Melissa Reynolds, PhD at Colorado State University (CSU) Department of Chemistry.

The purpose of this work is to investigate a novel strategy for preventing coagulopathic complications that occur during the use of extracorporeal life support (ECLS). This strategy involves a blood-compatible surface coating that is applied to the blood contacting surfaces in the ECLS device. The specific coating investigated is a copper containing metal organic framework (MOF) that catalyzes/accelerates nitric oxide (NO) release from bioavailable NO donors that are naturally present in the blood. As observed in natural vessels of the human body, NO prevents thrombus formation by inhibiting platelet activation and aggregation. Clinically, anti-clotting drugs are given to patients during ECLS therapy to prevent thrombus formation; however, these drugs are accompanied by a high risk of bleeding complications. Additionally, these drugs cannot be administered to trauma patients with pre-existing hemorrhagic complications, such as many combat casualties, that could otherwise benefit from ECLS support. This work aims to assess whether the NO-catalyzing MOF surface coating, which was developed in Dr. Reynold's lab at CSU, can prevent thrombosis and foreign surface-induced coagulopathy during ECLS without the use of anti-clotting drugs. To accomplish this, we will first develop methods to optimally apply this coating to ECLS circuits and test the efficacy and stability of the coating *ex vivo* once applied. We will then assess performance of the NO-generating circuits in healthy swine for 72 hours of ECLS therapy in an ICU setting. Finally, we will evaluate the NO-generating ECLS circuits in injured swine using a combat-relevant injury model of polytrauma (pulmonary contusion + hemorrhage). This phase of the study will also include a simulated aeromedical evacuation where subjects will be exposed to hypobaric pressures in an altitude chamber. Taken together, this project offers a comprehensive heparin free ECLS solution for both combat casualties and civilians, transported on ground, via air, or cared for in ICUs around the world.

2. **KEYWORDS:**

Acute respiratory distress syndrome, acute lung injury, extracorporeal life support, nitric oxide, coagulation

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Updates from the last semiannual report are highlighted below.

Specific Aim 1: Establish coating properties for the MOF ECLS circuitry					
Major Task 1: Coat catheters, tubing, and membranes with MOF	Months	Site 1	Site 2	% Complete	Status:
Objective 1.1: Develop polymer formulations of different MOF concentrations (0.1%, 0.5%, 1%, 5%)	1-3		Dr. Reynolds	100% (completed 01Feb19)	CSU developed multiple MOF formulations and developed process to synthesize large quantities of MOF to coat ECLS circuits for the study

Objective 1.2: Coat circuit components with MOF coatings (tubing, catheters, membranes)	1-3		Dr. Reynolds (100% Complete 15 Sept 21)	100% complete	CSU developed new method to apply MOF to 3/8" ID ECLS circuit tubing. CSU designed new method to assess solvent removal from ECLS tubing. NO gas to be utilized in lieu of MOF coating for membrane oxygenator in studies going forward.
Objective 1.3: Conduct physical testing to analyze adherence of MOF	1-3		Dr. Reynolds (100% Complete 15 Sept 21)	100% complete	CSU developed custom flow testing apparatus to evaluate tubing for coating delamination. CSU characterized the coated tubing under flow for adhesion (SEM), copper leaching (ICP-AES) and MOF activity (NOA analysis)
Objective 1.4: Conduct *ex vivo* testing to determine platelet adhesion to MOF coated components	1-3	Dr. Batchinsky (100% complete 15 Sept 20)	Dr. Reynolds (100% Complete 15 Sept 21)	100%	AREVA performed a second ex vivo circulation study using the new 3/8" coated MOF tubing prepared using new method developed at CSU. CSU conducted experiment to assess MOF activity following exposure of tubing to bovine plasma and conducted NOA analysis of tubing following flow testing with ovine blood.
Objective 1.5: Conduct *ex vivo* testing to determine NO release by MOF coated components	1-3	Dr. Batchinsky (100% complete 15 Sept 21)	Dr. Reynolds	100% complete	AREVA performed a second ex vivo circulation study using the new 3/8" coated MOF tubing prepared using new method developed at CSU. CSU measured MOF activity in post-circulation tubing. CSU will assess post-circulation tubing using SEM/ongoing.
Objective 1.6: Conduct testing to assess antithrombogenic effects of gaseous NO delivered in the ECLS membrane sweep gas	8-18	Dr. Batchinsky (100% Complete)		100% completed (Complete 15 Sept 20)	AREVA added MOF coating + NO gas group to ex vivo blood circulation study.
Milestone 1: Testing of MOF coating complete; coating process for Aims 2, 3 identified	18	Dr. Batchinsky	Dr. Reynolds	100% complete	MOF coating process complete for tubing. Attempts to coat membrane are unsuccessful to date, but we identified alternative solution to membrane coating by using NO gas delivered into membrane sweep gas.
Specific Aim 2: Determine device performance and systemic effects of MOF-coated ECLS circuitry in 72-hour *in vivo* experiment in uninjured swine					
Major Task 2: Regulatory Approval of Animal Use	Months	Site 1	Site 2	% Complete	Status:
Objective 2.1: Write animal use protocol; Obtain IACUC/ACURO approval	1-3	Dr. Batchinsky (12 swine, *sus scrofa*)		100% Complete (15 Mar 21)	Complete

Objective 2.2: Antimicrobial Assays and Cytotoxicity Study to Demonstrate the Cytocompatibility of MOF Coated ECLS Circuits (*ex vivo*)	6-9	Dr. Batchinsky and Hitesh Handa, PhD	Dr. Reynolds	100% (complete 01Oct19)	Performed static and dynamic bacterial adhesion assessment demonstrating that MOF coating reduces bacterial adhesion under both conditions.
Major Task 3: 72 hour studies of MOF coating in *uninjured* animals	Months	Site 1	Site 2	% Complete	Status:
Objective 3.1: Conduct animal studies comparing MOF coated ECLS tubing/catheters and membrane anticoagulation using gaseous NO vs. standard of care	3-18	Dr. Batchinsky		5% complete	With clear circuit coating strategy and characterization now complete at CSU, uninjured animal studies to start in October 2021. CSU has begun preparing circuits for animal testing.
Objective 3.2: Conduct NO release studies evaluating MOF-coated ECLS tubing with membrane anticoagulation using gaseous NO vs. standard of care *in vivo*	3-18	Dr. Batchinsky	Dr. Reynolds	5% complete	With clear circuit coating strategy and characterization now complete at CSU, uninjured animal studies to start in October 2021. CSU has begun preparing circuits for animal testing.
Milestone 2: Completion of testing in *uninjured* animals	18	Dr. Batchinsky	Dr. Reynolds	0%	N/A
Specific Aim 3: Evaluate the use of MOF-coated ECLS without systemic heparin *in vivo* in a combat relevant traumatic ARDS and aeromedical evacuation model					
Major Task 4: Regulatory Approval of Animal Use	Months	Site 1	Site 2	% Complete	Status:
Objective 4.1: Write animal use protocol; Obtain IACUC/ACURO approval	15-18	Dr. Batchinsky (24 swine, *sus scrofa*)		100% Complete (15 Mar 21)	Complete
Major Task 5: 72 hour studies of MOF coating in *injured* animals	Months	Site 1	Site 2	% Complete	Status:
Objective 5.1: Conduct animal studies comparing MOF coating vs. no MOF coating	18-36	Dr. Batchinsky		0%	N/A
Objective 5.2: Conduct NO release studies of MOF coated vs. no MOF coating *in vivo*	18-36	Dr. Batchinsky	Dr. Reynolds	0%	N/A
Milestone 3: Completion of testing in *injured* animals	36	Dr. Batchinsky	Dr. Reynolds	0%	N/A
Specific Aim 4: Evaluation of Coated Circuits following in-vivo testing					
Major Task 6: Thrombus deposition analysis after *in vivo* use	Months	Site 1	Site 2	% Complete	Status:
Objective 6.1: Collect, process, and analyze by SEM circuit and components after *in vivo* use	3-36	Dr. Batchinsky		0%	N/A

Objective 6.2: Conduct NO catalysis testing of circuit pieces after *in vivo* use	3-36		Dr. Reynolds	0%	N/A
Objective 6.3: Conduct structural stability testing of MOF structure after *in vivo* use	3-36		Dr. Reynolds	0%	N/A
Milestone 4: Completion of post use deposition and structural testing of circuit and components	36	Dr. Batchinsky	Dr. Reynolds	0%	N/A
Milestone 5: Completion of final reports and manuscripts	36	Dr. Batchinsky	Dr. Reynolds	0%	N/A

What was accomplished under these goals?

1) Major Activity 1: Coat ECLS catheters, tubing and membranes with MOF.

a) **Objective 1.1:** Develop polymer formulations of different MOF concentrations.

i) **Major activities:**

- Completed 01 Feb 2019 (See year 1 report)

ii) **Results/developments/achievements:**

b) **Objective 1.2:** Coat circuit components with MOF coatings (tubing, catheter, membrane).

i) Major activities:

- See Y3 Semiannual report for detailed summary of circuit coating modifications required to scale coating from ¼" ID circuit tubing to 3/8" ID circuit tubing.

ii) Results/outcomes/achievements:

- CSU recommends using 0.1% MOF formulation as detailed in Y3 semiannual report.

c) **Objective 1.3:** Conduct testing to analyze adherence of MOF on ECLS materials.

i) Major activities:

- CSU completed the 24- and 72-hour MOF stability testing under flow conditions. Following exposure to saline at 24- or 72- hours, delamination of the coating was analyzed using SEM imaging, ICP-AES was performed to assess copper leaching, and NO release testing was conducted to assess MOF activity.

ii) Results/outcomes/achievements:

- See Y3 Semiannual report for detailed diagram for flow testing setup.
  - 24 h flow experiments using 0.1% and 1% formulations (Table 1 Results):
    - Saline concentration: 0.9%
    - Flow rate: 2.5 L/min
    - Water bath: 38 °C
    - Tubing covered with foil to maintain temperature
    - 1/2' segment of coated tubing tested

Table 1. 24 h Flow Experiments

Formulation	n	Delamination	Cu Leaching
0.1%	3	No	No

1%	3	No	Yes

i. 72h flow experiments using 0.1% MOF formulation (Table 2 results):
- Saline concentration: 0.9%
- Flow rates (L/min): 1.0 (24 h), 2.0 (48 h), 2.5 (72 h)
- Water bath: 38 °C
- Tubing covered with foil to maintain temperature
- 3' segment of 0.1% coated tubing tested

Table 2. 72 h Flow Experiments

Formulation	n	Delamination	Cu Leaching	MOF Activity
0.1%	3	No	No	Yes

d) **Objective 1.4:** Conduct *ex vivo* testing of MOF-coated materials.

i) Major activities:

- AREVA team performed a second set of ex vivo circulation studies to evaluate the new 0.1% MOF coating formulation applied to the 3/8" ID tubing by CSU. (Results from the original ex vivo test using ¼" ID tubing reported in Y2 annual report).
- A total n=8 MOF tubing sets and n=8 CTRL tubing sets were tested using blood from swine donors. In this study, 1 meter length tubing segments are connected to a centrifugal pump on one end and a blood bag/reservoir on the other end (2 tubing pieces per tubing set). Blood is circulated through the loop at a flow rate of 1.5 Lpm. Heparin (UFH) is delivered to maintain the blood ACT at 125-160 sec throughout the 6-hour study. Blood is collected at start of circulation (baseline, BL), 3-hours (3H) and 6-hours (6H) after start of circulation for assessment of CBC, coagulation, platelet aggregation and activation, and to store plasma for assessment of NO metabolites. Tubing is collected at end of circulation to assess degree of blood deposition through visual observation and scanning electron microscopy imaging.

ii) Results/outcomes/achievements:

- Activated clotting time (ACT) was maintained in target range (125-160 seconds) with no significant difference in ACT, activated partial thromboplastin time (aPTT), total heparin infused and antithrombin III percent activity between groups (Table 3).
- No between groups results observed on CBC (Table 3)
- Methemoglobin (MetHb) levels were not elevated in either group and were not different between groups (Table 3).
- Free hemoglobin in plasma (PFHb) did not change over time and was not different between groups (Fig 01).
- There was no group difference in platelet count over time between groups. No group difference in platelet aggregation stimulated by ADP or COL was observed, although aggregation was reduced in all groups over time (not shown).
- P-selectin expression (expressed on activated platelets) was not significantly different between groups. Phosphatidyl Serine (PS) expression which signifies a pro-coagulant platelet state was not different between groups (see Figure 02).
- In the first iteration of this study using 1/4" circuit tubing and complete ECLS circuits with oxygenators, the MOF-coated circuitry performed similarly to the standard of care heparin-

coated circuitry (reported in Y2 Annual report). In this second iteration of the study using 3/8" ID circuit tubing connected to a centrifugal pump only (no oxygenator in line), again the coated tubing behaved similarly to the control uncoated tubing.

- CSU verified that MOF catalytic activity is still functional following 6 hours blood exposure (see Obj 1.5).

Value	Group	Baseline	3 Hour	6 Hour
ACT (sec)	CTRL	152 ± 6	139 ± 4	136 ± 3
	MOF	148 ± 5	141 ± 4	137 ± 2
aPTT (sec)	CTRL	52 ± 4	44 ± 3	48 ± 4
	MOF	55 ± 5	44 ± 4	49 ± 5
Total Heparin Infused (U)	CTRL	3 ± 3	78 ± 46	118 ± 50*
	MOF	6 ± 4	56 ± 31	87 ± 34*
Dimer (ug/mL)	CTRL	0.21 ± 0.03	0.22 ± 0.04	0.19 ± 0.03
	MOF	0.20 ± 0.04	0.22 ± 0.04	0.24 ± 0.04
RBC (x10^6 cells/µL)	CTRL	4.5 ± 0.3	4.5 ± 0.2	4.5 ± 0.3
	MOF	4.6 ± 0.3	4.5 ± 0.3	4.5 ± 0.3
WBC (x10^3 cells/µL)	CTRL	12 ± 1	11 ± 1*	11 ± 1*
	MOF	12 ± 1	11 ± 1*	11 ± 1*
MetHb (%)	CTRL	1.0 ± 0.1	1.2 ± 0.1	1.5 ± 0.0
	MOF	0.9 ± 0.1	1.3 ± 0.1	1.5 ± 0.0

Table 3. Blood parameters for swine donor blood circulated *ex vivo* through control (CTRL) ECLS circuit tubing (n=8) or metal organic framework coated (MOF) coated (using 0.1% CuBTTri formulation) circuit tubing (n=8). Blood samples were collected at initiation of circulation (BL), 3-hours and 6-hours after start of circulation. *Indicates significant within group difference from baseline value ($p<0.05$); †Indicates significant between group difference MOF vs CTRL ($p<0.05$).

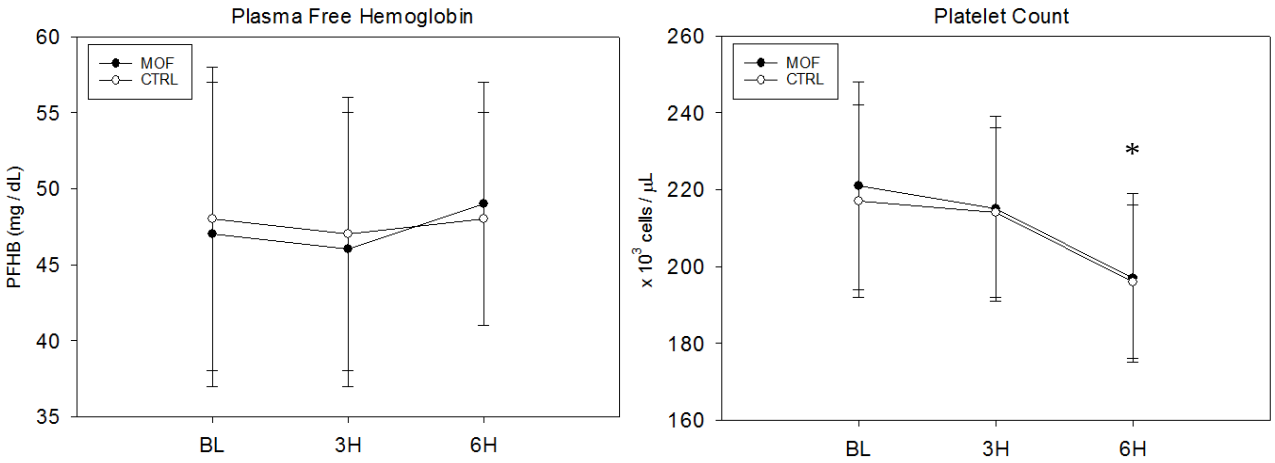

Fig 01. Plasma free hemoglobin (PFHb) concentration and absolute platelet count during 6 hours ex vivo blood circulation through standard/uncoated ECLS tubing (CTRL group) vs. MOF-coated tubing (MOF group). *Indicates significant change from baseline (BL) measurement MOF group ($p<0.05$).

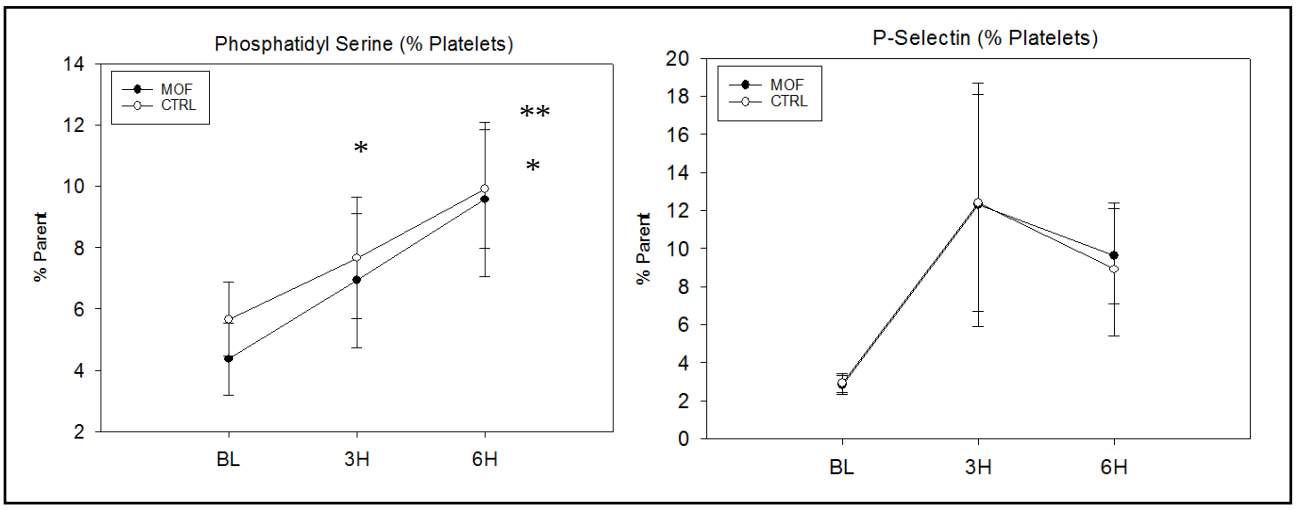

Fig 02. Phosphatidyl serine positive percentage of platelets (pro-coagulant platelets) and P-Selectin Positive platelet percentage (activated platelets) for 6 hours ex vivo blood circulation through uncoated ECLS circuit tubing (CTRL group) or metal-organic framework coated ECLS (MOF group). * Indicates significant difference within group from baseline (BL) value for MOF ($p<0.05$ for significance). **Indicates significant difference within group from baseline (BL) value for CTRL ($p<0.05$ for significance).

e) **Objective 1.5:** Conduct *ex vivo* testing to determine NO release of MOF-coated materials.

i) Major activities:

- AREVA shipped 3/8" ID tubing samples back to CSU following 6-hours ex vivo circulation testing using swine blood so that CSU could assess the catalytic activity of the coated tubing after blood exposure.
- CSU exposed MOF-coated tubing segments to bovine plasma and assessed MOF catalytic activity following exposure. Briefly, a 1cm tubing piece (control/no coating OR 0.1% MOF coated) was submerged in 20 mL bovine plasma for 2 hours (n=3/group) at 37 C and then evaluated for NO release.
- CSU performed *ex vivo* analysis where heparinized fresh ovine blood (used within 2 hours of collection, ACT>1000 sec) was circulated for 2 hours at 1.5 L/min using a test apparatus with 1-foot of 3/8" ID tubing using MOF-coated and CTRL tubing (n=3/group). Following circulation, tubing was tested for MOF catalytic activity.

ii) Results/outcomes/achievements:

- All MOF coated tubing demonstrated catalytic activity following the 6-hours *ex vivo* porcine blood study (Table 4).
- MOF tubing demonstrated catalytic activity following exposure to bovine plasma (Table 5).
- MOF tubing demonstrated catalytic activity following flow testing with ovine blood (Table 6).

Table 4: Post-*Ex vivo* MOF Activity			
Ex vivo Exp.	Tubing	n	MOF Activity

1	Control	4	Active ($p < 0.05$)
	MOF Coated	4	
2	Control	4	Active ($p < 0.05$)
	MOF Coated	4	
3	Control	4	Active ($p < 0.05$)
	MOF Coated	4	
4	Control	4	Active ($p < 0.05$)
	MOF Coated	4	

Table 5. Post-Plasma Exposure MOF Activity

Tubing	n	MOF Activity
Control	3	Active ($p < 0.05$)
MOF Coated	3	

Table 6. Post-Sheep Blood Flow MOF Activity

Tubing	n	MOF Activity
Control	3	Active ($p < 0.05$)
MOF Coated	3	

f) **Objective 1.6:** Conduct ex-vivo pilot testing to assess antithrombogenic effects of gaseous NO delivered in the ECLS membrane sweep gas.

i) Major activities:

- Objective Complete, results reported in Y1 Annual and Y2 Semi-Annual reports.

ii) Results/outcomes/achievements:

- Objective Complete.

2) <u>Major Activity 2</u>: Regulatory approval of animal use to determine device performance and systemic effects of MOF-coated ECLS circuitry in 72-hour *in vivo* experiment in uninjured swine.

a) **Objective 2.1:** Write animal use protocol; obtain IACUC/ACURO approval.

i) **Major activities:**

- Contract negotiations between UTSA and Geneva/AREVA completed. Amendment to animal use protocol to enable use of the NO gas generator in lieu of MOF coating application to membrane oxygenators was approved by UTSA an ACURO.
- Animal studies to be initiated in October 2021 now that CSU has finalized 3/8" tubing preparation methods and characterization; and Aim 1 Objectives have been completed.

i) **Results/developments/achievements:**

- AREVA is prepared to start animal studies immediately now that a resolution to the 3/8" circuit tubing issue has been resolved and the new coating method has been fully validated in *in vitro* and *ex vivo* test methods at AREVA and CSU.

b) **Objective 2.2:** Antimicrobial Assays and Cytotoxicity Study to demonstrate cytocompatibility of MOF-coated ECLS circuits (*ex vivo*).

i) **Major activities:**
- Objective completed 01 Oct 2019.

ii) **Results/developments/achievements:**
- Results reported in Y1 Annual Report.

3) <u>Major Activity 3:</u> Determine device performance and systemic effects of MOF-coated ECLS circuitry in 72-hour *in vivo* experiment in uninjured swine.

a) Objective 3.1: Conduct animal studies comparing MOF coated ECLS tubing and membrane anticoagulation using gaseous NO versus standard of care.

i) Major activities:
- AREVA trained all personnel on operation of NO gas generator.
- AREVA trained all personnel on animal handling, care, and blood sample collection for this protocol.
- AREVA trained all personnel on ECLS system operation and management for animal studies.
- CSU has synthesized several batches of custom MOFs for both circuit preparation, and currently they have enough MOF prepared to make 148 0.1% CuBTTri 3/8" tubing segments. 3 of 6 total circuits required for uninjured animal study have been prepared. The second batch of 3 circuits will be prepared once the first few animal studies are completed to ensure there are no unanticipated issues.

ii) Results/developments/achievements:
- CSU and AREVA are prepared to start animal studies ASAP in the next reporting period (October 2021).

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

This project has allowed for several laboratory technicians at AREVA who are currently applying to medical school to learn to operate clinical coagulation and hematology equipment through the ex vivo circulation studies performed at AREVA. Personnel were also trained to operate ECLS systems during the ex vivo study and in preparation for the *in vivo* study.

Dr. Teryn Roberts in Dr. Batchinsky laboratory contributed to this project during her doctoral training with Dr. Batchinsky as her dissertation research mentor. Following receiving her PhD degree, she has been intimately involved in all aspects of the study and has been promoted to a Co-PI position on the project.

How were the results disseminated to communities of interest?
The results were disseminated at key medical conferences including invited lectures by Dr. Roberts at the ASAIO annual meeting ("Coagulation management approaches for extracorporeal pulmonary support in the pre-hospital setting") and at the 35th Annual Meeting of the Japanese Association for the Surgery of Trauma ("Bio-inspired surfaces and regional anticoagulation strategies for extracorporeal life support: material assessment and development protocol for clinical translation"). The results of the NO generator testing were selected for presentation at the 2021 MHSRS meeting which was unfortunately cancelled.

The AREVA and CSU teams are now beginning to draft a manuscript summarizing their ex vivo circulation testing and 3/8" tubing coating methods for publication.

What do you plan to do during the next reporting period to accomplish the goals?
At CSU, the team will continue to prepare circulation tubing for the animal studies. Additionally, after each animal experiment, the AREVA team will ship tubing samples collected at the end of 72-hours blood exposure back to CSU for complete characterization of coating function and stability.

At AREVA, the 72-hour uninjured animal test phase will begin in October. Following completion of n=12 uninjured studies (6 animals per group), the injury test phase will begin.

Geneva and CSU will collaborate to finalize a manuscript summarizing SEM analysis methods for biomaterial studies. The teams will begin to draft manuscripts detailing the tubing coating, characterization, and ex vivo circulation testing now that they have been completed.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?
In this reporting period, CSU developed a novel method for application of a NO-generating MOF to 3/8" ID ECLS circuit tubing. These methods could be utilized to apply the MOF coating to other indwelling medical devices, catheters, pacemakers and stents and/or to apply other coatings to ECLS circuitry during a wide range of therapeutic applications from dialysis to ECLS. At AREVA, the *ex vivo* ECLS circulation system and panel of methods we have developed to assess blood viability and platelet activation have established a new test-bed for ECLS systems and blood-compatible coatings that emphasizes the importance of clinically-relevant flow conditions and utilizes clinical test protocols to make the results relevant and translatable to clinicians and clinician scientists.

What was the impact on other disciplines?
The unexpected complications with application of the coating to the membranes as well as the investigation to resolve this challenge will provide a wealth of knowledge to materials scientists, chemists, and bioengineers. Additionally, the *ex vivo* testing system we developed in this study will inform materials scientists/bioengineers of the clinical conditions under which biomaterials for ECLS need to be tested. The latter may have significant applications to the field of medical device development and potentially could change the FDA regulations for anticoagulant solutions in critical care. The experimental test bed developed by us also serves as a benchmark platform for analysis of

blood biocompatibility by materials scientists and biomedical engineers. This project aims to inform the basic scientists and engineers that develop these biocompatible materials of the specific clinical considerations required for materials that are designed for ECLS applications.

What was the impact on technology transfer?
Nothing to report, work ongoing.

What was the impact on society beyond science and technology?
Development of blood-compatible coating for ECLS (and other medical applications) would revolutionize clinical care and change clinical practice guidelines. Specifically, for acute lung injury and ARDS improving the safety of ECLS will open a range of therapeutic options in a clinical population in need of alternative care solutions other than mechanical ventilation which can exacerbate lung injury and may be ineffective in most severely injured. This could also enable development of home-use/portable/out of hospital/wearable devices for chronic lung support or those awaiting transplant.

5. **CHANGES/PROBLEMS:**
CSU experienced significant delays when it was unexpectedly determined that all aspects of the ECLS tubing coating process had to be redesigned for the larger 3/8" ID tubing (all original work was performed using the ¼" ID tubing). Further, the core facility at CSU led to delays in solvent measurement testing, so the Reynold's team developed a HS GC method to analyze samples themselves within 30 minutes. CSU also experienced issues in developing a solvent removal process for the 3/8" tubing. They tried 8 different methods and identified a method going forward that requires 2-3 weeks for solvent removal per tube. Equipment delays also caused issues including a GC software issues (required 5 days internal resolution and an on-site visit with Agilent and a follow-on call to resolve the issue) and issues with the nitric oxide analyzer (NOA) (ozone generator on NOA stopped working, the instrument had to be sent out for repairs). CSU also experienced delay during ovine blood studies as the CSU Vet school experienced complications when collecting the sheep blood using heparin. Different heparin administration methods and blood bags were utilized to resolve the issue. Ultimately, CSU was able to get 3 successful collections for the blood flow experiments.

The delays encountered at CSU for 3/8" tubing preparation and characterization caused the AREVA team to repeat the ex vivo test that was performed for the original MOF coating formulation (1.0% MOF on ¼" ID tubing) using the new coating formulation (0.1% MOF on 3/8" ID tubing). The AREVA team could not begin animal studies until the new MOF formulation was fully validated and characterized at CSU. Dr. Batchinsky and AREVA team met with interim science officer Dr. Maria Disotuar Hidalgo and new project science officer Ms. Swapna Sista on 26 March 2021 to detail the problems encountered and the team's actions to resolve. An NCE was filed and approved to accommodate COVID-19 related project delays, as well as the delays in material preparation and characterization detailed above. We are now on track to start animal studies ASAP (October 2021).

Changes in approach and reasons for change
Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to Report.

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.

6. **PRODUCTS:**

Publications, conference papers, and presentations

Journal publications.

Thai, JE, Tuttle RR, DeRoo J, Cuchiaro JH, Reynolds MM. Three-layer, bottom-up synthesis of catalytically active Cu-MOF nanosheets. *Submitted to Applied ACS Nanomaterials.*

Tuttle RR, Daly RE, Rithner CD, Reynolds MM. Monitoring metal-organic framework catalysis *in vitro:* determination of the stoichiometry and rate of CuBTTri catalyzed NO generation from S-nitrosoglutathione directly in blood plasma. *Submitted to Inorganic Chemistry Frontiers 2021.*

Roberts TR, Choi J, Wendorff DS, Harea GT, Beely BM, Sieck KS, Douglass ME, Singha P, JB Dean, Handa H, Batchinsky AI. Evaluation of tethered-liquid perfluorocarbon coating for heparin-free extracorporeal life support: a 72-hour intensive care unit study. *ASAIO J.* 2021; 67(7):798-808.

Zang Y, Roberts TR, Batchinsky AI, Reynolds MM. Metal-organic framework polymer coating inhibitis *Staphylococcus aureus* attachment on medical circulation tubing under static and dynamic flow conditions. *ACS Appl. Bio Mater.* 2020; 3(6): 3535-3543.

Roberts TR, Garren M, Handa H, Batchinsky AI. Toward an artificial endothelium: development of blood-compatible surfaces for extracorporeal life support. *J Trauma Acute Care Surg.* 2020; S2(89): 59-68.

Batchinsky AI, Wendorff DS, Jones J, Beely BM, Roberts TR, Choi J, Harea GT, Cancio LC, Davis M, Cannon J, Sams V. Noninvasive SpO2/FiO2 ratio as a surrogate for PaO2/FiO2 ratio during simulated prolonged field care and ground and high-altitude evacuation. *J Trauma Acute Care Surg.* 2020; S2(89): 126-131.

Roberts TR, Harea GT, Singha P, Sieck KN, Beely BM, Wendorff DS, Choi JH, Ande S, Handa H, Batchinsky AI. Heparin-Free Extracorporeal Life Support Using Tethered Liquid Perfluorocarbon: A Feasibility and Efficacy Study. *ASAIO J.* 2020; 66(7): 809-817.

Books or other non-periodical, one-time publications.
Nothing to report this period.

Other publications, conference papers and presentations.

Invited Conference Lecture: Roberts TR, Batchinsky AI. Bio-inspired surfaces and regional anticoagulation strategies for extracorporeal life support: material assessment and development protocol for clinical translation. *35th Annual Meeting of the Japanese Association for the Surgical Trauma (Virtual).* 28 May 2021.

Invited Conference Lecture: Roberts TR, Batchinsky AI. Coagulation management approaches for extracorporeal pulmonary support in the pre-hospital setting. *ASAIO 66th Annual Conference (Virtual);* 4 June 2021, Washington D.C., USA.

Conference Presentation: Zang Y, Oliva L, Roberts R, Ande S, Reynolds MM, Batchinsky AI. Quantitative analysis of digital vs scanning electron microscopy images as an assessment tool fo post-extracorporeal life support clot formation evaluation. *37th Annual Children's National Symposium ECMO and the Advanced Therapies for Cardiovascular and Respiratory Failure (Virtual).* 21-23 February, 2021.

Conference Presentation: Oliva L, Zang Y, Roberts R, Ande S, Reynolds MM, Batchinsky AI. Evaluation of post-extracorporeal life support clot formation using scanning electron microscopy and digital imaging. *University of the Incarnate Word School of Medicine Research Day (Virtual).* April, 2021.

University Seminar Presentation: Zang Y. Medical device induced thrombosis and infections: what should we do? *University of the Incarnate Word School of Pharmacy.* 15 July 2021.

Website(s) or other Internet site(s)
Nothing to report this period.

Technologies or techniques:
Technique: application of CuBTTri/MOF coating to 7 ft ECLS tubing (1/4" ID and 3/8" ID).

Technique: application of CuBTTri/MOF coating to dual-lumen Avalon ECLS catheter.

*both techniques will be shared in manuscripts describing ECLS/MOF coating process and evaluation (manuscripts in preparation).

Inventions, patent applications, and/or licenses
Nothing to report this period.

Other Products
Nothing to report this period.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Andriy I. Batchinsky, MD
Project Role:	Principal Investigator
Researcher Identifier:	0000-0001-8601-2827
Nearest Person Month Worked:	0.9
Contribution to Project:	Overseeing and carrying out the project protocol, collecting and analyzing data, preparing and finalizing manuscripts and reports.

Name:	Teryn Roberts, PhD
Project Role:	Co-Investigator
Researcher Identifier:	0000-0002-2460-6432
Nearest Person Month Worked:	2.2
Contribution to Project:	Carrying out the project protocol, collecting and analyzing data, and preparing and finalizing manuscripts and reports.

Name:	Jae Choi, PhD
Project Role:	Co-Investigator
Researcher Identifier:	N/A
Nearest Person Month Worked:	1.4
Contribution to Project:	Carrying out the project protocol, carrying out pathological assessment and assays, and preparing manuscripts and reports.

Name:	Brendan Beely
Project Role:	Research Coordinator
Researcher Identifier:	N/A
Nearest Person Month Worked:	1.4
Contribution to Project:	Performing routine laboratory procedures, assisting with study protocols, and preparing reports.

Name:	Dan Wendorff
Project Role:	Laboratory Manager
Researcher Identifier:	N/A
Nearest Person Month Worked:	1.9
Contribution to Project:	Performing routine laboratory procedures, preparation of animal protocol and oversight of IACUC review, overseeing Lab. Techs.

Name:	George Harea
Project Role:	Research Associate
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.9

Contribution to Project:	Assisting with protocols, assisting with biochemical assays, assisting in data collection and management, assisting in the preparation of manuscripts and reports.

Name:	John Jones
Project Role:	Statistician
Researcher Identifier:	N/A
Nearest Person Month Worked:	1.5
Contribution to Project:	Assisting with data organization, statistical analyses including power analysis, and data interpretation, as well as contributing to manuscripts and reports.

Name:	Isabella Garcia
Project Role:	Laboratory Technician
Researcher Identifier:	N/A
Nearest Person Month Worked:	2.7
Contribution to Project:	Assisting with data collection and technical procedures. Assist with preparations for animal testing. Will assist with large animal handling.

Name:	Ji Lee
Project Role:	Laboratory Technician
Researcher Identifier:	N/A
Nearest Person Month Worked:	1.4
Contribution to Project:	Assisting with ex vivo experiment execution, maintenance of coagulation devices and data collection.

Name:	Hailee Alaniz
Project Role:	Laboratory Technician
Researcher Identifier:	N/A
Nearest Person Month Worked:	2.1
Contribution to Project:	Assisting with data collection and technical procedures. Assist with preparations for animal testing. Will assist with large animal handling.

Name:	Clayton Smith
Project Role:	Laboratory Technician
Researcher Identifier:	N/A
Nearest Person Month Worked:	1.7
Contribution to Project:	Assisting with data collection and technical procedures. Assist with preparations for animal testing. Will assist with large animal handling.

Name:	Brittney Lewis
Project Role:	Regulatory Specialist
Researcher Identifier:	N/A
Nearest Person Month Worked:	1.2
Contribution to Project:	Assisting with the drafting and review of technical documents such as manuscripts and reports and completing literature reviews.

Name:	Robert Willis
Project Role:	Laboratory Technician
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.4
Contribution to Project:	Assisting with data collection and technical procedures. Assist with preparations for animal testing. Will assist with large animal handling.

Name:	Yanyi Zang
Project Role:	Postdoctoral Fellow
Researcher Identifier:	N/A
Nearest Person Month Worked:	3.1
Contribution to Project:	Assisting ex vivo circulation study execution and post-circulation material analysis, biosample processing

Name:	Zachary Allen
Project Role:	Laboratory Technician
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.3
Contribution to Project:	Assisting with data collection and technical procedures. Assist with preparations for animal testing. Will assist with large animal handling.

Name:	Cameron Chan
Project Role:	Summer Intern
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.2
Contribution to Project:	Assist with ex vivo circulation studies. Post-circulation tubing imaging and analysis. Scanning electron microscopy sample preparation and imaging.

Name:	Kaitelynn Beely
Project Role:	Summer Intern
Researcher Identifier:	N/A
Nearest Person Month Worked:	0.2

Contribution to Project:	Assist with ex vivo circulation studies. Post-circulation tubing imaging and analysis. Scanning electron microscopy sample preparation and imaging.

Name:	Melissa Reynolds
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.75
Contribution to Project:	Management and oversight of the project such as supervise research, administrate research grant, mentor and supervise graduate research assistant and undergraduate researchers; experimental design

Name:	Alyssa Melvin
Project Role:	Graduate Research Assistant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	8.5
Contribution to Project:	Conducts all NOA experiments, SEM analysis, GC analysis, coating of tubing, troubleshoot experiments, experimental design, GSNO synthesis and characterization

Name:	Robert Tuttle
Project Role:	Graduate Research Assistant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	4
Contribution to Project:	Synthesized MOF, studied mechanism of MOF interaction with GSNO.

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Name:	Jon Thai
Project Role:	Graduate Research Assistant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	8.5
Contribution to Project:	Scaled up and synthesized ligand; synthesized CuBTTri derivative that is more catalytically active.

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

N/A

What other organizations were involved as partners?

N/A

8. **SPECIAL REPORTING REQUIREMENTS**

Collaborative Awards

None. CSU report is included in this report.

Quad Charts

N/A

9. **APPENDICES**

Appendix A – Award Chart