AWARD NUMBER: W81XWH-19-1-0210

TITLE: Cell-Specific microRNA Regulation of Ventilation-Induced Lung Injury During Hemorrhagic Shock

PRINCIPAL INVESTIGATOR: Samir Ghadiali

CONTRACTING ORGANIZATION: The Ohio State University, Columbus, OH

REPORT DATE: August 2021

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2021	Annual	01Jul2020-30Jun2021

4. TITLE AND SUBTITLE

Cell-Specific microRNA Regulation of Ventilation-Induced Lung Injury During Hemorrhagic Shock

5a. CONTRACT NUMBER
W81XWH-19-1-0210

5b. GRANT NUMBER
GRANT12663206

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Samir Ghadiali

E-Mail: ghadiali.1@osu.edu

5d. PROJECT NUMBER
PR181466

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

The Ohio State University,
1960 Kenny Rd. Columbus, OH
43210

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 acute respiratory distress syndrome (ARDS) is a life-threatening condition that affects critically ill patients, especially soldiers who develop hemorrhagic shock (HS). Although these patients frequently require life support with mechanical ventilation (MV), the mechanical forces generated during ventilation can cause de novo injury known as ventilator induced lung injury (VILI). Since the molecular mechanisms responsible for VILI are not known, there are presently no effective pharmacological therapies for HS induced ARDS. Recently, microRNAs (miRNAs) have emerged as important small molecule regulators of gene expression and several miRNAs (miR-146a and miR-155) are dysregulated during ARDS. However, it is not known if therapeutic modulation miR-146a or miR-155 expression can be used to regulate the mechanotransduction processes that lead to VILI. The overall goal of this Discovery Award proposal is to demonstrate the therapeutic potential of miR-146a and miR-155 in reducing VILI in HS induced ARDS.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	13	USAMRDC

TABLE OF CONTENTS

1. **INTRODUCTION:**

The overall goal of our Discovery Award proposal is to determine the therapeutic potential of gene regulatory molecules (miR-146a and miR-155) to reduce ventilator induced lung injury (VILI) during the acute respiratory distress syndrome (ARDS) subsequent to hemorrhagic shock and resuscitation (HSR). The specific purpose of this research is to determine how modulating miR-146a and/or miR-155 expression in different cell types can be used to regulate lung injury/inflammation during mechanical ventilation (MV) and we proposed two specific aims as the scope of this project. First, we will use a "humanized" lung-on-a-chip system to investigate how modulating miR-146a and miR-155 expression in specific cell types alters HSR and MV induced lung injury. Second, we will use a novel nanotechnology delivery system to modulate miR-146a and miR-155 expression in mouse models of HSR/ARDS and investigate if targeted over-expression of these molecules in specific cell types can be used to reduce VILI. Our expectation is that these studies will yield important insights into how miR-146a and miR-155 can be used to regulate HSR and mechanically-induced lung injury and inflammation and will lay the foundation for future pre-clinical studies that use miR-146a and miR-155 to regulate HSR and MV induced ARDS in both large animal models and ex-vivo human models.

2. **KEYWORDS:** Acute Respiratory Distress Syndrome, Ventilation Induced Lung Injury, Nanoparticles, microRNAs, organ-on-a-chip, Mechanical Ventilation, Oxidative Stress, Hemorrhagic Shock

3. **ACCOMPLISHMENTS:**

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

Major Goal 1: Determine how cell-specific modulation of miR-146a and miR-155 expression alters lung injury and inflammation in a novel lung-on-a-chip model of VILI during HSR induced ARDS.

Subtask 1a: Use a humanized lung-on-a-chip model to simulate the ischemia/reperfusion that occurs during HSR and the mechanical forces associated with VILI (i.e. volutrauma and atelectrauma). Incorporate human alveolar macrophages. **Timeline: complete by month 4. Status: 100% Completed**

Subtask 1b: Evaluate how HSR and mechanical forces influence cell viability, alveolar-capillary permeability and inflammatory signaling. **Timeline: complete by month 6. Status: 100% Completed**

Subtask 2: Investigate how over-expression/silencing of miR-146a and miR-155 in multiple cell types alters HSR and mechanically induced injury and inflammation. **Timeline: complete by month 12. Status: 100% Completed**

Subtask 3: Determine the molecular mechanisms and targets by which these miRs regulate injury/inflammation. **Timeline: complete by month 18. Status: 100% Completed**

Major Goal 2: Determine the therapeutic potential of over-expressing/silencing miR-146a and miR-155 in an in-vivo model of VILI during HSR induced ARDS.

Subtask 1: Use wild-type C57BL/6J mice to investigate how global over-expression and silencing of miR-146a or miR-155 alters HSR and ventilation induced lung inflammation, mechanics and injury. **Timeline: complete by month 6. Status: 100% Completed**

Subtask 2a: Use novel nanotechnology-based delivery systems to deliver pre and anti miR-146a constructs to multiple cell types in wild-type mice. Simulate HSR induced ARDS and VILI. **Timeline: complete by month 10. Status: 100% Completed** with the caveat that anti miR studies were not conducted because only overexpression (i.e. pre miR studies) was shown to be effective in reducing HSR induced ARDS and VILI in-vitro.

Subtask 2b: Use novel nanotechnology-based delivery systems to deliver pre and anti miR-155 constructs to multiple cell types in wild-type. Simulate HSR induced ARDS and VILI. **Timeline:**

complete by month 14. Status: 0% Completed Note: In order to maximize the clinical impact of this grant, we have chosen to focus on miR-146a over-expression only since this method has proven to be the most reliable and robust way to regulate VILI. This is subtask, which focused on the in-vivo effects of miR-155, has been abandon to allow us to devote resources and time to in-vivo miR-146a studies.

Subtask 3: Use novel nanotechnology-based delivery systems to deliver combined pre-miR146a to macrophages and anti-miR-155a to lung epithelial and endothelial cells. Simulate HSR induced ARDS and VILI. **Timeline: complete by month 18. Status: 50% Completed** Note: as per above, in-vivo studies are now focusing on miR-146a over-expression only, and instead of delivering anti-miR155 to epithelial/endothelial cells we are focusing on investigating if delivery of pre-miR-146a to activated macrophages alone is more effective than global delivery to all cell types.

What was accomplished under these goals?

As reported in last year's annual technical report, we made significant progress in year 1 on both major goals of this grant and many of our accomplishments have been published at a very high impact publication in Nature Communications (*Bobba et al. Nat Commun 12, 289 (2021)*). In the past year we have made additional progress on both aims/goals and we provide a brief summary of these activities below.

Major Goal 1: Determine how cell-specific modulation of miR-146a and miR-155 expression alters lung injury and inflammation in a novel lung-on-a-chip model of VILI during HSR induced ARDS.

Subtask 1a: Use a humanized lung-on-a-chip model to simulate the ischemia/reperfusion that occurs during HSR and the mechanical forces associated with VILI (i.e. volutrauma and atelectrauma). Incorporate human alveolar macrophages.

As reported in our year 1 progress report we have developed a novel lung-on-a-chip platform that can simulate both the injurious mechanical forces that occur during VILI and the oxidative stress conditions that occur during HSR. We have used this system to simulate the oxidative stress that occurs during HSR and have also incorporated human alveolar macrophages into this system. The major finding from these studies is a robust lung-on-a-chip platform that can recapitulate HSR conditions therefore be used to investigate how HSR and mechanical forces influence cell injury and inflammation (see subtask 1b below). An additional novel finding from these studies is that macrophage-epithelial interactions exacerbate mechanically induced injury during VILI and we have identified the biomechanical and molecular mechanism as described below.

Subtask 1b: Evaluate how HSR and mechanical forces influence cell viability, alveolar-capillary permeability and inflammatory signaling.

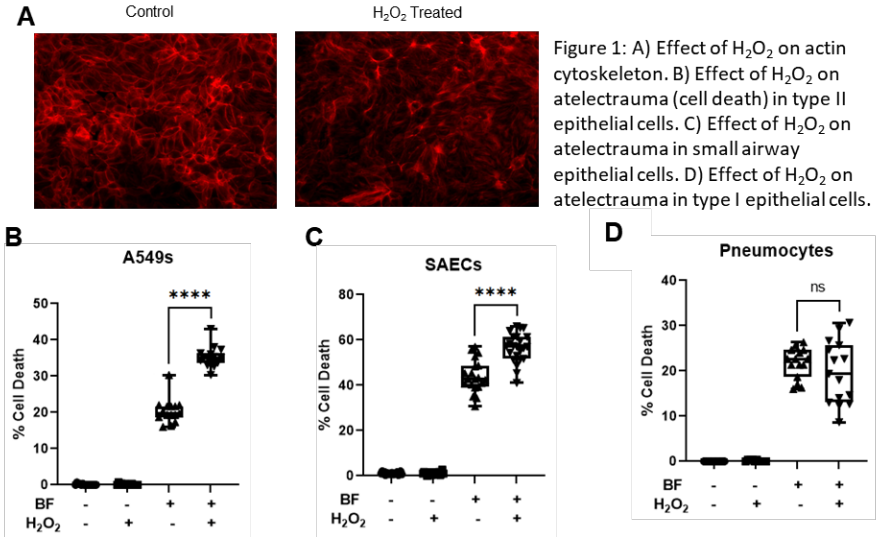

Figure 1: A) Effect of H_2O_2 on actin cytoskeleton. B) Effect of H_2O_2 on atelectrauma (cell death) in type II epithelial cells. C) Effect of H_2O_2 on atelectrauma in small airway epithelial cells. D) Effect of H_2O_2 on atelectrauma in type I epithelial cells.

As reported in our year 1 progress report we have demonstrated that oxidative stress associated with HSR leads to an alteration in cell morphology and cytoskeletal structure which makes epithelial cells more susceptible to atelectrauma. In year 2 we have extended these studies to better understand the mechanisms by which HSR conditions exacerbate the cell injury that occurs during mechanical ventilation. First, we demonstrated that oxidative stress, which is simulated using hydrogen peroxide (H_2O_2) treatment, results in cytoskeletal disruption (Fig 1A) and a softer cell. Computational simulations predict that these mechanical changes are responsible for the increased injury observed in cells undergoing oxidative stress and atelectrauma conditions. Interestingly, we also found that type 2 alveolar epithelial cells (Fig 1B) and small airway epithelial cells (Fig 1C) both exhibited increased cell death during atelectrauma when treated with H_2O_2. However, type 1 alveolar epithelial cells (Fig 1D) did not experience increased cell death during atelectrauma when treated with H_2O_2. Type 1 cells exhibit a flatter, spread morphology while type 2 and small airway cells are taller and more rounded. Computational simulations indicated that these difference in morphology are responsible for the differential injury response during atelectrauma and oxidative stress. We are currently using our system to investigate how these oxidative stress conditions alter alveolar-capillary permeability and inflammatory signaling.

Subtask 2: Investigate how over-expression/silencing of miR-146a and miR-155 in multiple cell types alters HSR and mechanically induced injury and inflammation.

As reported in our year 1 progress report we have demonstrated that overexpression of miR-146a is effective at reducing the increased inflammatory signaling that occurs during mechanical ventilation. We specifically demonstrated that over-expressing miR-146a in either epithelial cells or macrophages can reduce mechanically-induced inflammation and refer the reader to our year 1 report and Nature Communication publication for details. In year 2, we extended these studies to investigate how over-expressing miR-146a influences mechanical injury and cell death during ventilation. Interestingly, as shown in Fig 2AB, we found that over-expressing miR-146a lead to an increase in cell injury in

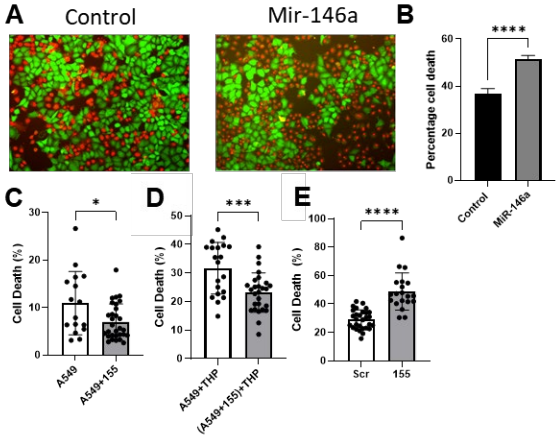

Figure 2: AB) Effect of Mir-146a overexpression on cell death during atelectrauma (red=dead cells). C) Effect of miR-155 overexpression on atelectrauma (cell death) in monocultures of epithelial cells. D) Effect of over-expressing miR155 in epithelial cells only on atelectrauma in epithelial-macrophage co-cultures. E) Effect of over-expressing miR155 in both epithelial cells and macrophages on atelectrauma.

epithelial cells during atelectrauma. This data suggests that although miR-146a is effective at reducing mechanically induced inflammation, miR-146a over-expression in epithelial cells may exacerbate mechanically induced injury. We had previously demonstrated that overexpressing miR-155 in alveolar epithelial cells results in a significant reduction in atelectrauma. In year 2 we conducted additional studies to investigate the cell-specific mechanisms of miR-155 cyto-protection during atelectrauma. As shown in Figure 2C, consistent with previous studies, over-expressing miR-155 in epithelial cells reduced atelectrauma injury in monocultures. In addition, over-expressing miR-155 in epithelial cells alone (not macrophages) lead to reduced cell injury in a co-culture model (Fig 2D). Interestingly, over-expressing miR-155 in both epithelial cells and macrophages exacerbated epithelial cells death during atelectrauma (Fig 2E) and we are currently investigating if miR-155 over-expressing in macrophages alone exacerbates epithelial cell injury. One hypothesis of why miR-155 over-expression in macrophages exacerbates epithelial cell injury during atelectrauma is that miR-155 is pro-inflammatory in macrophages and alters epithelial-macrophage interactions. In support of this hypothesis, in year 1 we demonstrated that inhibiting epithelial-macrophage interactions at gap-junctions reduces the degree of atelectrauma and in year 2 we have started to investigate how other integrin interactions regulate macrophage-induced epithelial cell injury during atelectrauma.

Subtask 3: Determine the molecular/biomechanical mechanisms and targets by which these miRs regulate injury/inflammation.

In year 1, we explored the molecular mechanisms by with miR-146a and mir-155 regulate injury/inflammation and in year 2 we extended these studies to investigate the possible biomechanical mechanisms by which miR-155 regulates cell injury during atelectrauma. We discovered that miR-155 overexpression results in an altered morphology and are currently using computational techniques to determine if this alteration in morphology can explain why miR-155 treated cells are protected from injury during atelectrauma.

Major Goal 2: Determine the therapeutic potential of over-expressing/silencing miR-146a and miR-155 in an in-vivo model of VILI during HSR induced ARDS.

As stated in the last year's annual report, the major objective for the current reporting period was to initiate the in-vivo HSR model of ARDS. Importantly, in the 1st year of funding we established an in-vivo model of HSR in mice and obtained the equipment necessary for this experiment. Unfortunately, this in-vivo model was established by Research Associate that left during year 1 in part due to a COVID related shutdown of the lab. However, in year 2 of this grant we were able to hire a new Research Associate, Emily Sholasky, who had the necessary background to execute the HSR protocol and complete the in-vivo subtasks under goal 2. Importantly, the no-cost extension was critical in providing this research associate the time needed to learn a complicated surgical protocol and re-establish this model in our lab.

Subtask 1: Use wild-type C57BL/6J mice to investigate how global over-expression and silencing of miR-146a or miR-155 alters HSR and ventilation induced lung inflammation, mechanics and injury.

As described in our previous report, we have used wild type and miR-146a knock out mice to demonstrate that miR-146a over-expression can be used to mitigate VILI. This groundbreaking study published in Nature Communications (*Nat Commun 12, 289 (2021)*) was the first to demonstrate that microRNAs and nano-technology delivery platforms can be used to regulate/mitigate VILI. In the past year, we have re-developed an animal model system that can be used to test the efficacy of this microRNA therapeutic in a military relevant model, HSR induced ARDS. In this model, mice are anesthetized, and the right and left femoral arteries are catheterized. The left catheter is used for monitoring blood pressure while the right is used to conduct a controlled bleed. Blood is withdrawn over a 15 min interval until the blood pressure reaches 30-40 mmHg and this pressure was maintained for either 15, 35 or 45 minutes. The mouse was then resuscitated over a 15-minute interval with a transfusion of shed blood and lactated ringers' solution. Finally, a tracheostomy was performed, and the mouse was subjected to an injurious mechanical ventilation protocol as described previously (*Bobba et al. Nat Commun 12, 289 (2021)*). An iStat blood analyzer was used to monitor blood gases and analytes and a flexivent was used to monitor changes in lung mechanics and injury.

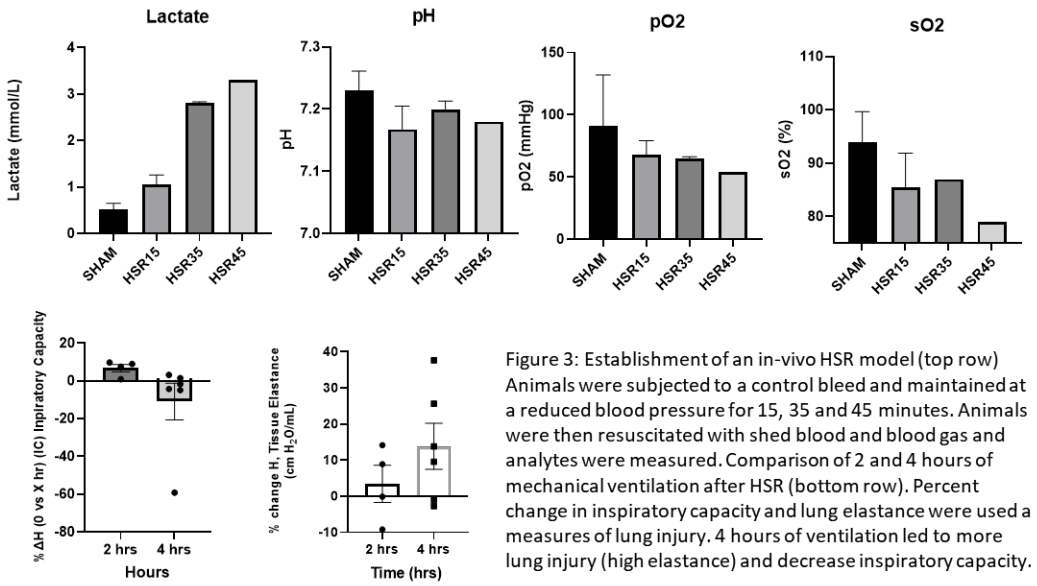

Figure 3: Establishment of an in-vivo HSR model (top row) Animals were subjected to a control bleed and maintained at a reduced blood pressure for 15, 35 and 45 minutes. Animals were then resuscitated with shed blood and blood gas and analytes were measured. Comparison of 2 and 4 hours of mechanical ventilation after HSR (bottom row). Percent change in inspiratory capacity and lung elastance were used a measures of lung injury. 4 hours of ventilation led to more lung injury (high elastance) and decrease inspiratory capacity.

As shown in Figure 3 (top row), this in-vivo model of HSR causes significant alterations in lactate level and blood oxygenation. Specifically, longer durations of shock cause progressively larger increases in lactate level and reductions in oxygen saturation. Lower pH was observed at all durations of shock and lower pO2 levels were also observed at all levels of shock. We have also investigated how 2 or 4 hours of mechanical ventilation following this in-vivo model of HSR alters lung physiology and injury. As shown in Figure 3 (bottom row), 4 hours of ventilation lead to a significant reduction in inspiratory capacity as well as an increase in lung elastance. Note these ventilation studies were done after only 15 minutes of shock in our HSR model. To maximize our efforts in the remaining 2 months of this grant, we will focus only on how over-expressing miR-146a alters the degree of VILI during HSR using this newly established model and will also focus on using 4 hours of ventilation and 15 minutes of shock.

Subtask 2a: Use novel nanotechnology-based delivery systems to deliver pre and anti miR-146a constructs to multiple cell types in wild-type mice. Simulate HSR induced ARDS and VILI.

As reported in our Nature Communication publication and in our year 1 progress report, we have developed and used a novel solid lipid nanoparticle formulation to dramatically over-express miR-146a by ~100-1000 fold in wild-type mice. Importantly, co-localization analysis indicated that ~44% of the nanoparticles were delivered to epithelial cells while ~52% were delivered to alveolar macrophages and we were thus able to deliver miR-146a to multiple cell types. Treatment with miR-146a nanoparticles dampened inflammation, significantly reduced alveolar-capillary permeability and significantly improved lung compliance during mechanical ventilation. These improvements in inflammation and lung mechanics resulted in improved blood oxygenation, a key clinical outcome. We were also able to demonstrate that the mechanisms responsible for miR-146a's therapeutic effect was its targeting and downregulation of TRAF6 and SMAD4. Interestingly, miR-146a treatment also resulted in less neutrophil infiltration, another hallmark of VILI and ARDS. These **exciting and groundbreaking findings** are the first to show that pulmonary delivery of miR-146a loaded lipid nanoparticles can be used to potently increase miR-146a levels and that this therapeutic increase in microRNA expression mitigates lung injury during injurious mechanical ventilation. As noted below, now that our HSR induced ARDS model has been reestablished, we plan to investigate if this nanotechnology-based gene delivery platform can be used to regulate VILI during HSR as well as in other models of ARDS.

Subtask 2b: Use novel nanotechnology-based delivery systems to deliver pre and anti miR-155a constructs to multiple cell types in wild-type. Simulate HSR induced ARDS and VILI. **Timeline: complete by month 14. Status: 0% Completed** This is subtask, which focused on miR-155, has been abandoned to allow us to devote resources and time to miR-146a.

As noted above, we have for the time being decided to focus on miR-146a overexpression since this microRNA has shown tremendous therapeutic potential. While we are still interested in investigating the effect of miR-155 over-expression, in-vitro studies using our lung-on-a-chip platform indicate that highly

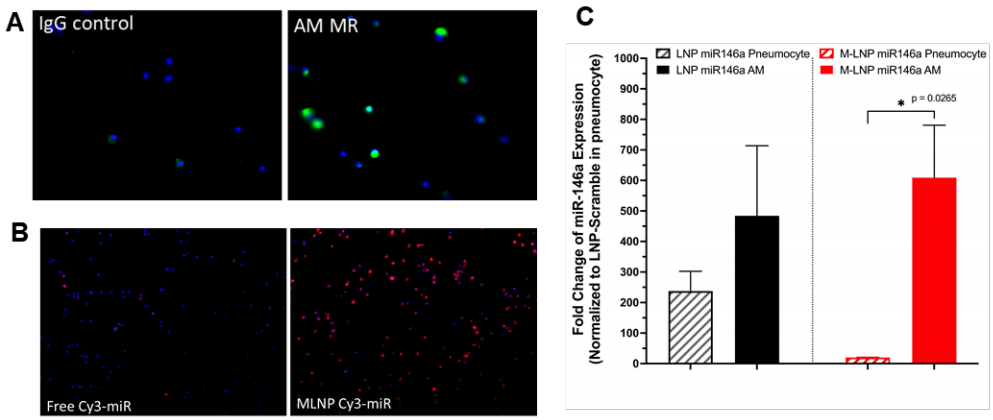

Figure 4: Development of an alveolar macrophage (AM0 targeted nanoparticle system for miR146a delivery. A) activated alveolar macrophages express the mannose receptor (green=mannose, blue=nuclei). B) A mannosylate lipid nanoparticles containing a fluorescent Cy3-miR shows significant uptake by nearly 100% of macrophages (red stain). C) miR-146a expression in epithelial/pneumocytes and AMs after treatment with a non-targeted lipid nanoparticle (LNP) and a mannosylated lipd nanoparticle (M-LNP).

accurate cell specific delivery of this pleotropic microRNA might be necessary in order to regulate/mitigate VILI during HSR induced ARDS. Therefore, we have focused our energy and remaining funds on optimizing our cell-specific gene delivery platform (see subtask 3 below).

Subtask 3: Use novel nanotechnology-based delivery systems to deliver combined pre-miR146a to macrophages and anti-miR-155a to lung epithelial and endothelial cells. Simulate HSR induced ARDS and VILI. **Timeline: complete by month 18. Status: 50% Completed** Note: as per above, in-vivo studies are now focusing on miR-146a over-expression only, and instead of delivering anti-miR155 to epithelial/endothelial cells we are focusing on investigating if delivery of pre-miR-146a to activated macrophages alone is more effective than global delivery to all cell types.

The main hypothesis to be tested in this subtask was that cell specific delivery and over-expression of miR-146a may be needed to optimally regulate VILI during HSR induced ARDS. This hypothesis is supported by data obtained in year 2 which indicates that miR-146a over-expression in epithelial cells might exacerbate cell death during atelectrauma (see Fig 2) and therefore specific delivery of miR-146a constructs to alveolar macrophages might be needed/warranted. In order to test this hypothesis, it is critical to develop a targeted nanoparticle formulation and demonstrate that this delivery method only over-expresses the microRNA of interest in the cell of interest (i.e. macrophages). In year 2 we have made major progress on developing this targeted nanoparticle system. First, to target alveolar macrophages, we have modified our nanoparticles to incorporate a mannose motif on the particle surface. As shown in Figure 4A, we have demonstrated that activated alveolar macrophages express mannose at a sufficient quantity and that macrophages treated with our mannose conjugated nanoparticle exhibit nearly 100% particle uptake (Fig 4B). To determine if this increased uptake translates into increased expression in macrophages only, we loaded pre-miR-146a constructs into our mannosylated nanoparticle (M-LNP) and treated co-cultures of epithelial cells and macrophages with both these miR loaded M-LNPs as well as miR loaded non-targeted nanoparticles (LNP). Cells were then sorted using a flow cytometry protocol that was developed by our lab over 6 months. PCR was used to quantify miR-146a expression in the different cell types and as shown in Figure 4C, although the miR-146a expression was similar in both cells when using the nontargeted LNPs, treating co-cultures with the miR-146a loaded M-LNP lead to very high miR expression only in macrophages. This data strongly demonstrates that our novel targeted nanoparticle formation preferentially delivers microRNAs to macrophages only. We therefore now have a selective way to over-express miR-146a in macrophages and our plans (see below) call for using this cell-targeted delivery platform to test the hypothesis that selective over-expression of miR-146a in activated macrophages is more effective at reducing VILI during HSR induced ARDS than global over-expression.

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

Nothing to Report

- **How were the results disseminated to communities of interest?**

We have presented our results at the 9th International Biofluids Conference and the Biomedical Engineering Societies annual conference. We have also published results in Nature Communications (*Nat Commun 12, 289 (2021)*).

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

We are currently in the final months of this grant and our plans are to use our non-targeted and targeted microRNA delivery system to test the efficacy of cell-specific delivery in an HSR-induced ARDS model. For these studies, we have the HSR model as well as the subsequent VILI models in-hand and have demonstrated the targeting capabilities of our delivery system. We therefore, do not anticipate any problems completing this study. We also plan to submit an additional 2-3 publications describing the findings obtained in this study.

4. **IMPACT:**

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

The major impact of this project to date on the field of ARDS and VILI is the development of a novel nanoparticle-based delivery platform that can dramatically over-express microRNAs and thereby significantly downregulate lung inflammation and injury during mechanical ventilation. As such, we have for the first time developed a potential pharmaceutical way to prevent VILI. However, ongoing experiments are critical to demonstrate the potential of this miR therapeutic in military relevant model of ARDS, i.e. HSR-induced ARDS.

- **What was the impact on other disciplines?**

Nothing to report

- **What was the impact on technology transfer?**

Nothing to Report

- **What was the impact on society beyond science and technology?**

The development of a microRNA based therapeutic for ARDS has the potential to have major impacts on society. In particular, patients with severe COVID-19 infections suffer from ARDS and require mechanical ventilation. As with other forms of ARDS, there is currently no pharmaceutical way to prevent VILI in these patients. This research program has developed a nanotechnology platform that can deliver therapeutic levels of microRNAs to specific cells within the lung and has the potential to provide an important new way to regulate VILI in COVID-19 patients. We are therefore planning preliminary experiments to investigate if miR-146a can regulate mechanically-induced lung injury subsequent to COVID-19 infection. If these results are positive, we plan to apply for additional federal funding to further develop this therapeutic approach.

5. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change

As noted above, our in-vitro studies indicated that the pleotropic nature of miR-155 would make designing appropriate in-vivo studies difficult and due to the limited time available in the no-cost extension, we have abandoned the in-vivo miR-155 studies.

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

In March of 2020, due to COVID-19 related restrictions, we were required to shut down our laboratory and significantly restrict access to the labs for several months. After the shutdown, social distancing and reduced density requirements only allowed us to return to ~50% capacity in the summer with slightly more activity in the fall. As a result, we were not able to initiate several of the planned animal experiments in specific aim/goal 2. However, we were able to maintain our colonies of miR 146a KO mice as well as other

animal colonies during the COVID shutdown and were able to fully develop our HSR protocol (including acquisition and testing of all needed equipment) before the COVID shutdown. As a result, the research associate who was to originally complete several animal studies in the summer was not able to do so and this individual entered medical school in fall. In year 2, we hired a new research staff member who has all the skills needed to complete the in-vivo HSR studies. We have also experienced delays due to 1) equipment failure (breaking of the catheters used during the HSR studies) and 2) a shutdown of the facility that was providing us the polyurethane polymer fibers for our ventilator-on-a-chip monitoring system. We have 1) obtained new equipment and are making sure that all personnel are well trained to avoid additional failures and 2) have formed a collaboration with a material scientist at OSU who routinely fabricates polymer fiber meshes and are currently fabricating the required meshes in her laboratory.

Changes that had a significant impact on expenditures

Due to the need for new staff to complete the planned in-vivo animal studies, the expenditures for staff are larger than anticipated. We will accommodate this by reducing other salaries such that the planed work can be completed with no additional funds in the no-cost extension.

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

None

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals.

None

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS:

"Nanoparticle delivery of microRNA-146a regulates mechanotransduction in lung macrophages and mitigates lung injury during mechanical ventilation", Christopher Bobba, Qinqin Fei, Vasudha Shukla, Hyunwook Lee, Pragi Patel, Rachel K Putman, Carleen Spitzer, MuChun Tsai, Mark D. Wewers, John W. Christman, Megan N. Ballinger, Samir Ghadiali, Joshua A. Englert.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Samir Ghadiali*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-6845-774X Web of Science ResearcherID: C-5463-2012
Nearest person month worked:	*2.4*

Contribution to Project:	*Dr. Ghadiali has managed all aspects of the research program, designed experiments, conducted data analysis, submitted animal and human subject protocols, wrote manuscripts related to the funded work and reported results to funding agencies.*
Funding Support:	

○

Name:	*Joshua Englert*
Project Role:	*Co-PI*
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-1257-2239
Nearest person month worked:	*0.6*
Contribution to Project:	*Dr. Englert has managed and help design all of the animal experiments, conducted data analysis, assisted with animal and human subject protocols and wrote manuscripts related to the funded work.*
Funding Support:	*NIH RO1*

Name:	*Emily Sholasky*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	None
Nearest person month worked:	*12*
Contribution to Project:	*Ms. Sholasky was responsible for re-establishing the in-vivo model of HSR and conducting all in-vivo experiments associated with this project.*
Funding Support:	

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

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. **SPECIAL REPORTING REQUIREMENTS**

○ **COLLABORATIVE AWARDS:** N/A

○ **QUAD CHARTS:** *N/A*

9. **APPENDICES:** *None*