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TITLE: Mitochondrial Transplantation: A Novel Therapy for Lung Fibrosis

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CONTRACTING ORGANIZATION: Methodist Hospital Research Institute, Houston, TX

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Chronic lung diseases such as idiopathic pulmonary fibrosis (IPF) are prevalent among veterans and U.S. military personnel. IPF is a progressive, irreversible, and lethal disease with no effective treatment save for lung transplantation. IPF arises from relentless and extensive fibroproliferative injury, which itself stems from the inability of normal repair processes in the lung to deactivate following stimuli. The disease is characterized by focal zones of fibroblast proliferation. Transforming growth factor-β (TGF-β) plays a crucial role in fibrosis development, mediating cell activation, migration, and invasion. Increased TGF-β mediates metabolic reprogramming in cells involved in IPF progression, shifting bioenergetics from oxidative phosphorylation (OXPHOS) towards glycolysis. In fibroblasts and myofibroblasts, TGF-β increases expression of glycolytic enzymes and glucose transporters, as well as lactate production. In alveolar epithelial type II (AE2) cells, genes involved in metabolism are downregulated, and there is increased lactate production. A more glycolytic phenotype is also observed in alveolar macrophages in IPF. Decreased ATP production occurs in fibroblasts and myofibroblasts, while in IPF AE2 cells, fibroblasts, and macrophages, decreased electron transport chain (ETC) complex activity and lower oxygen consumption rates (OCR) have been observed. This work aims to deliver polymer-functionalized mitochondria to fibroblasts, AE2 cells, and macrophages in IPF lungs with the goal of restoring a favorable metabolic phenotype that can attenuate or reverse the disease. Our previous findings highlight that TGF-β treatment of fibroblasts resulted in metabolic reprogramming towards glycolysis and a pro-fibrotic phenotype. Herein, we show that mitochondria coated with a Dextran-triphenlyphosphonium (Dextran-TPP) polymer were able to decrease the expression of glycolytic enzymes and reduce the expression fibroblast-to-myofibroblast differentiation and epithelial-mesenchymal transition (EMT) markers in fibroblasts and alveolar epithelial cells. We also demonstrate that Dextran-TPP mitochondria administered to a bleomycin mouse model of IPF has significant therapeutic effects on pulmonary vasculature. Findings highlight a novel therapeutic strategy targeting dysregulated metabolism in IPF.

15. SUBJECT TERMS
Idiopathic pulmonary fibrosis, pulmonary fibrosis, fibroblasts, bioenergetics, mitochondria, metabolism, transforming growth factor-beta, glycolysis, oxidative phosphorylation, alveolar epithelial cells, alveolar macrophages

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Table of Contents

1. INTRODUCTION

Chronic lung disease is one of the most prevalent diseases among veterans and U.S. military personnel deployed to Iraq and Afghanistan. An increasing trend of incidence was found between interstitial lung diseases, including idiopathic pulmonary fibrosis (IPF), and deployment-related exposures such as dust and sandstorms, as well as industrial fires. IPF is a progressive, irreversible, and lethal disease, with patients dying within 2-5 years after diagnosis. At present, there is no effective treatment for IPF, save for lung transplantation. IPF arises from relentless and extensive fibroproliferative injury that occurs when normal repair processes in the lung are unable to deactivate following stimuli. One of the pathophysiological hallmarks of IPF is fibroblast foci, or focal zones of fibroblast proliferation. Transforming growth factor-β (TGF-β) plays a crucial role in development of fibrosis, mediating cell activation, migration, and invasion. Importantly, increased TGF-β mediates metabolic reprogramming in IPF lungs, with specific cell types exhibiting a metabolic shift from oxidative phosphorylation (OXPHOS), the highly efficient method of ATP production, towards a less efficient process of glycolysis, despite an adequate supply of oxygen. In fibroblasts and myofibroblasts, TGF-β increases expression of glycolytic enzymes and glucose transporters, with findings showing heightened lactate production as well. In alveolar epithelial type II (AE2) cells, genes involved in lipid synthesis and metabolism are downregulated, and there is increased lactate production. A more glycolytic phenotype is also observed in alveolar macrophages in IPF. Decreased ATP production occurs in fibroblasts and myofibroblasts, while in IPF AE2 cells, fibroblasts, and macrophages, decreased electron transport chain (ETC) complex activity and lower oxygen consumption rates (OCR) has been observed. The proposed work aims to deliver polymer-functionalized mitochondria to fibroblasts, AE2 cells, and macrophages in IPF lungs with the goal of restoring a favorable metabolic phenotype that can attenuate or reverse the disease. Our first aim involved evaluating the capacity of bioengineered mitochondria to restore cellular energetics in IPF fibroblasts, AE2 cells, and macrophages, while our second aim involved determining whether bioengineered mitochondrial transplantation can treat experimental IPF.

2. KEYWORDS

Idiopathic pulmonary fibrosis, pulmonary fibrosis, fibroblasts, bioenergetics, mitochondria, metabolism, transforming growth factor-beta, glycolysis, oxidative phosphorylation, alveolar epithelial cells, alveolar macrophages

3. ACCOMPLISHMENTS

What were the major goals of the project?

SA 1 - Evaluate the capacity of bioengineered mitochondria to restore cellular energetics in IPF fibroblasts, AE2 cells, and macrophages

Major Task 1: Cellular uptake examination in cells

Milestone 1: Relative quantification of mitochondria uptake in the different cell lines compared to non-coated mitochondria
Proposed completion date: month 26
Percentage of completion: 66%

Milestone 2: IACUC approval for *in vivo* experiments
Proposed completion date: month 3
Percentage of completion: 100% (completion in month 7)

Major Task 2: Bioenergetic and mitochondrial functional analysis
Milestone: Improved bioenergetic changes following mitochondrial transplantation into different pulmonary cells
Proposed completion date: month 30
Percentage of completion: 50%

Major Task 3: Cell proliferation, migration, morphology examination
Milestone: Reduction in TGF-β stimulated proliferation and migration in cells following mitochondrial transplantation
Proposed completion date: month 27
Percentage of completion: 50%

Major Task 4: Expression of fibrotic markers
Milestone: Demonstration of reduced expression of pro-fibrotic genes in cells following treatment with mitochondria
Proposed completion date: month 26
Percentage of completion: 90%

SA 2 - Determine whether bioengineered mitochondrial transplantation can treat experimental IPF

Major Task 5: Biodistribution examination in BLM model of fibrosis in mice
Milestone: Increased uptake of polymer functionalized mitochondria in lungs compared to uncoated mitochondria; increased uptake of mitochondria in lungs in diseased vs healthy lungs
Proposed completion date: month 27
Percentage of completion: 90%

Major Task 6: Efficacy evaluation in BLM model of lung fibrosis in mice
Milestone: Efficacious attenuation of disease progression, markedly reduced pro-fibrotic mediators after intravenous administration of polymer functionalized mitochondria
Proposed completion date: month 30
Percentage of completion: 35%

What was accomplished under these goals?

Our goal was to metabolically reprogram various cell types in IPF lungs. We hypothesized that mitochondrial delivery to fibroblasts, AE2 cells, and alveolar macrophages would properly regulate cellular bioenergetics, preventing disease progression in a mouse model of IPF.

Previously, we synthesized a dextran-triphenylphosphonium (TPP) (Dextran-TPP, **Fig. 1a**) polymer conjugate to functionalize isolated

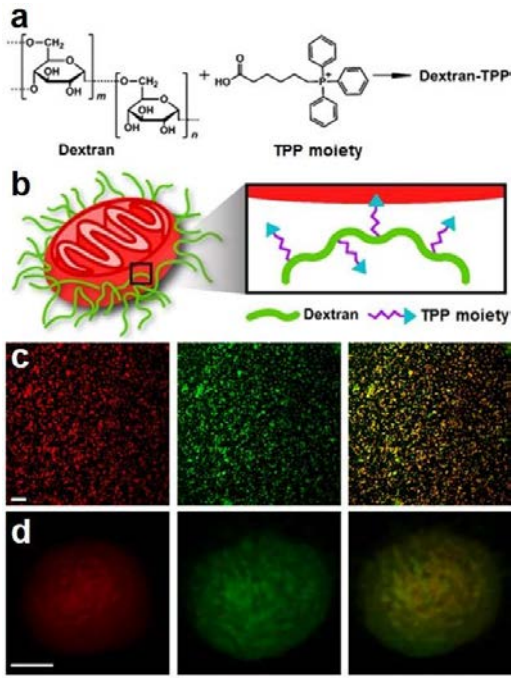

Figure 1. Dextran-TPP coating of isolated mitochondria. **a**) Chemical structures of dextran and TPP. **b**) Schematic of a Dextran-TPP coated mitochondrion, highlighting TPP incorporation into the mitochondrion. **c**) Confocal microscopy of Dextran-TPP coated mouse liver-derived mitochondria. Dextran-TPP was labelled with FITC (green) and mitochondria labelled with MitoTracker Deep Red (red). Scale bar = 20 μm. **d**) Magnification of coated mitochondrion. Scale bar = 0.5 μm.

mitochondria (**Fig. 1b**). The polymer conjugate was found to comprehensively coat isolated mitochondria (**Fig. 1c, d**), had higher uptake in cancer and cardiac cells compared to uncoated mitochondria, and triggered a bioenergetic switch in breast cancer cells and cardiomyocytes. Upon examination of the average basal oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), a significant enhancement of OXPHOS and shift away from a glycolytic phenotype was observed following transplantation of Dextran-TPP mitochondria to cells.

In the previous reporting period, our results showed that TGF-β stimulation contributed to mitochondrial dysfunction and glycolytic reprogramming of human lung fibroblasts (MRC-5 cells). TGF-β stimulation in these cells increased HIF-1α and 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) expression, the latter an enzyme responsible for increased rate of glycolysis. TGF-β treatment decreased ATP production, increased glucose consumption, and resulted in an increase in the ECAR/OCR ratio – indicative of a glycolytic phenotype. Consequently, MRC-5 cells stimulated with TGF-β had upregulated Smad signaling and increased expression of fibronectin and α-SMA. These data showed that TGF-β stimulation of MRC-5 cells resulted in glycolytic reprogramming of fibroblasts and an activated fibroblast phenotype. One goal we have not met is to determine the effect of TGF-β stimulation on fibroblast migration and proliferation. Another goal we have not met is to establish the conditions for TGF-β stimulation of alveolar macrophages (Subtask 1, Major Task 1).

In the previous reporting period, uptake of Dextran-TPP mitochondria (DTM) in fibroblasts (MRC-5) and alveolar epithelial cells (A549) was corroborated by flow cytometry and confocal microscopy. The goals that we have not met is uptake and internalization of Dextran-TPP mitochondria in alveolar macrophages (Subtask 2, Major Task 1).

Mitochondrial transplantation to fibroblasts and alveolar epithelial cells was expected to metabolically reprogram TGF-β-stimulated cells. Transplantation of Dextran-TPP mitochondria was to produce a shift from a glycolytic phenotype towards enhanced OXPHOS. Previously, the effect of Dextran-TPP mitochondria transplantation on TGF-β-stimulated MRC-5 cell bioenergetics was examined, highlighting a dose-dependent increase in OCR and ATP production. Bioenergetic analysis (e.g. Seahorse evaluation) after mitochondrial treatment (Subtask 4, Major task 2) in alveolar epithelial cells and macrophages remains to be completed. In the current reporting period, we demonstrated that Dextran-TPP mitochondria treatment of TGF-β-stimulated MRC-5 cells resulted in a decrease in the glycolytic enzymes PFKFB3 and hexokinase II (HKII) (**Fig**. **2**), signaling a decrease in the rate of glycolysis. Following TGF-β stimulation, Smad2 and Smad3 become phosphorylated, and SMAD-2/3 activation regulates pro-fibrotic gene expression, including collagens, proteoglycans, and matrix metalloproteases (MMPs), to name a few. In

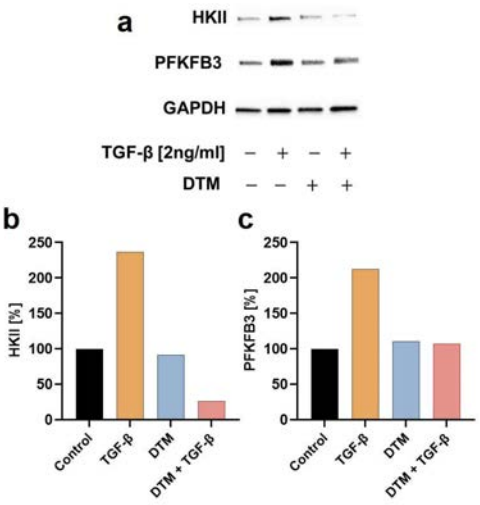

Figure 2. Dextran-TPP mitochondria attenuated glycolytic enzyme expression in TGF-β-treated fibroblasts. MRC-5 cells were incubated with TGF-β (2 ng/ml), Dextran-TPP mitochondria (DTM, 5 μg/ml) and DTM+TGF-β and protein expression levels of HKII and PFKFB3 were detected by Western blot at 24 h **(a)**. **b)** and **c)** represent quantification of expression normalized to β-actin and relative to Control.

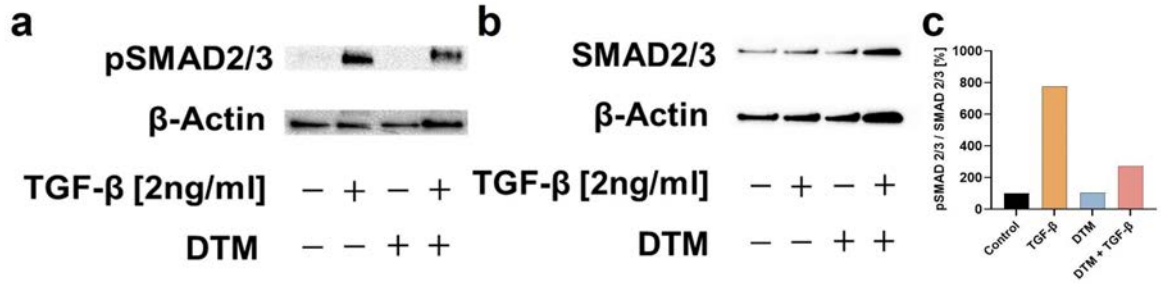

Figure 3. DTM decreased Smad signaling in TGF-β-treated MRC-5 cells. MRC-5 cells were incubated with TGF-β (2 ng/ml), DTM (5 µg/ml) and DTM+TGF-β and expression of pSMAD 2/3 and SMAD 2/3 detected by Western blot at 24 h. **(a)** and **(b)**. **c)** Quantification of expression normalized to β-actin and relative to Control.

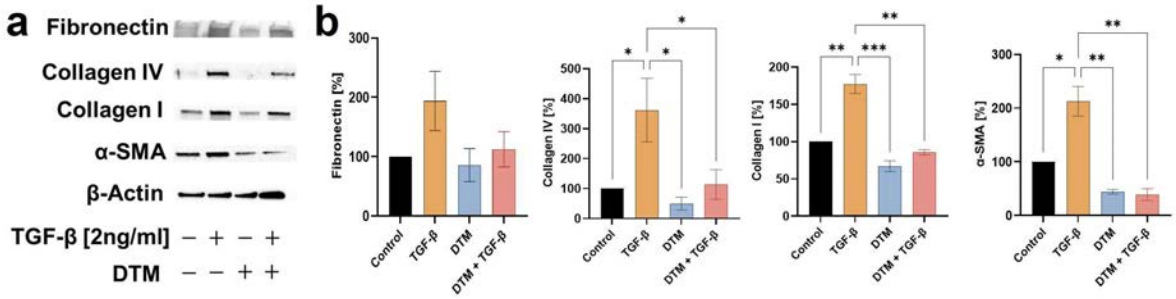

Figure 4. DTM attenuated principal fibroblast-to-myofibroblast differentiation markers in TGF-β-stimulated fibroblasts. MRC-5 cells were incubated with TGF-β (2 ng/ml), DTM (5 µg/ml) and DTM+TGF-β, and expression levels of fibronectin, collagen IV, collagen I, α-SMA were evaluated by Western blot at 24 h **(a)**. **b)** Quantification of expression normalized to β-actin and relative to Control. Data presented as mean ± SEM of at least three separate experiments. Statistical analysis was performed using two-way ANOVA followed by Tukey's multiple comparison test. *P≤0.05, **P≤0.005, and ***P≤0.002 among means was considered statistically significant.

the current reporting period, we demonstrated that Dextran-TPP coated mitochondria treatment of TGF-β-stimulated MRC-5 cells had reduced Smad signaling compared to TGF-β-stimulated MRC-5 cells (**Fig**. **3**), hinting at a robust anti-fibrotic response.

In the current reporting period, the effect of Dextran-TPP coated mitochondria transplantation on an activated fibroblast phenotype, as well as expression of fibroblast-to-myofibroblast differentiation markers, was examined. Unmodulated myofibroblasts destroy normal lung architecture through excessive extracellular matrix (ECM) deposition. As can be seen in **Figure 4**, Dextran-TPP coated mitochondria to TGF-β-treated MRC-5 fibroblasts resulted in decreased expression of α-SMA, a protein indicative of a myofibroblast phenotype. Moreover, Dextran-TPP coated mitochondria treatment of MRC-5 cells stimulated with TGF-β decreased fibronectin, a glycoprotein that facilitates fibroblast attachment to the ECM. Lastly, transplantation of Dextran-TPP coated mitochondria to TGF-β-stimulated MRC-5 cells decreased the expression of type I and IV collagens compared to TGF-β-treated MRC-5 fibroblasts. This work corresponds to Subtask 7 in Major Task 4. The goals we did not meet in the current reporting period were the evaluation of cell proliferation and migration of TGF-β-stimulated fibroblasts treated with Dextran-TPP coated mitochondria (Subtask 6, Major task 3)

In the current reporting period, the effect of Dextran-TPP coated mitochondria transplantation on the glycolytic phenotype of TGF-β-stimulated alveolar epithelial cells (A549) was examined. TGF-β-induced epithelial-mesenchymal transition (EMT) is associated with trans-differentiation that correlates with metabolic reprogramming (glycolytic induction) and suppressed mitochondrial

function. Alveolar epithelial cells that undergo EMT contribute to pulmonary fibrosis by promoting a pro-fibrotic microenvironment and activation of fibroblasts through paracrine signaling. As can be seen in **Figure 5**, and contrary to what was observed in MRC-5 fibroblasts, TGF-β treatment did not increase the expression of the glycolytic enzymes PFKFB3 and HKII. This is due to A549 cells being adenocarcinomic, which has previously been shown to be more glycolytic than non-tumorigenic alveolar epithelial cells. Thus, A549 cells are known to have higher glucose uptake and lactate production and have lower OCR than other alveolar epithelial cells. Consequently, TGF-β stimulation of A549 cells did little to alter the glycolytic flux of A549 cells. Importantly, our findings demonstrate that Dextran-TPP coated mitochondria treatment of A549 cells decreased the expression of PFKFB3 and HKII. TGF-β treatment of A549 cells did, however, influence Smad signaling. As can be seen in **Figure 6**, TGF-β stimulation led to a 2-fold increase in SMAD-2/3 activation. Importantly, following treatment of TGF-β-stimulated A549 cells with Dextran-TPP coated mitochondria, Smad signaling decreased in these cells.

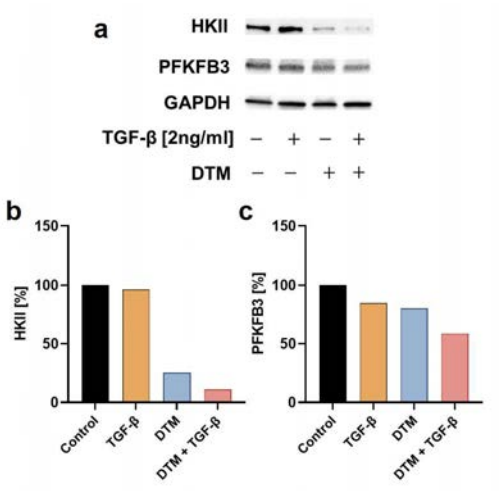

Figure 5. Dextran-TPP mitochondria attenuated glycolytic enzyme expression in TGF-β-treated alveolar epithelial cells. A549 cells were incubated with TGF-β (2 ng/ml), Dextran-TPP mitochondria (DTM, 5 µg/ml) and DTM+TGF-β and protein expression levels of HKII and PFKFB3 were detected by Western blot at 24 h **(a)**. **b)** and **c)** represent quantification of expression normalized to β-actin and relative to Control.

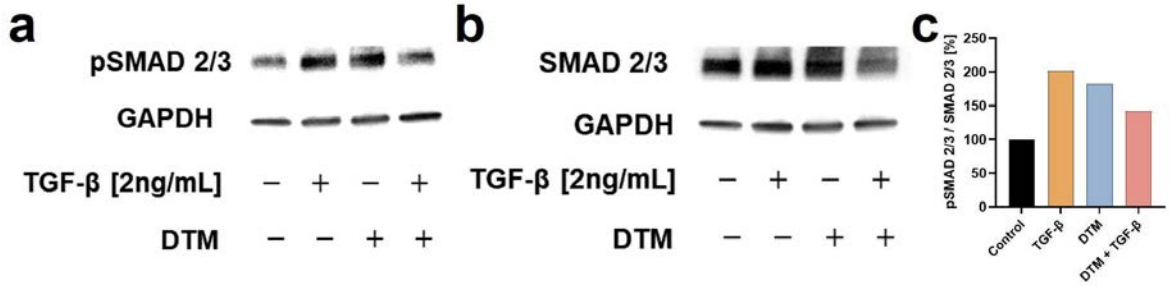

Figure 6. DTM decreased Smad signaling in TGF-β-treated alveolar epithelial cells. A549 cells were incubated with TGF-β (2 ng/ml), DTM (5 µg/ml) and DTM+TGF-β and expression of pSMAD 2/3 and SMAD 2/3 detected by Western blot at 24 h. **(a)** and **(b)**. **c)** Quantification of expression normalized to β-actin and relative to Control.

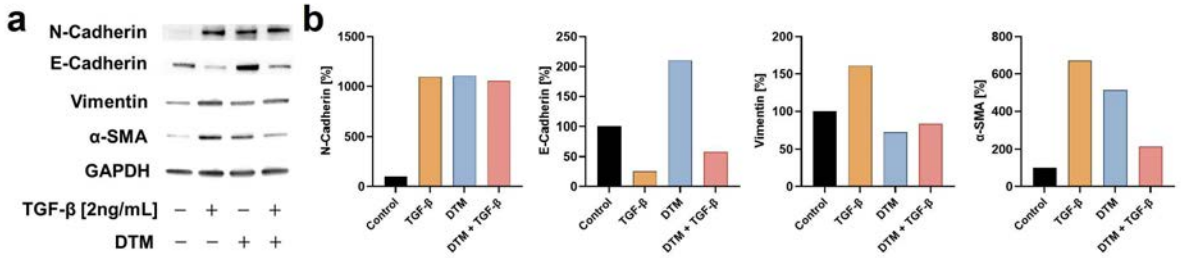

Figure 7. DTM effects on epithelial and mesenchymal cell markers in TGF-β-stimulated alveolar epithelial cells. A549 cells were incubated with TGF-β (2 ng/ml), DTM (5 µg/ml) and DTM+TGF-β, and expression levels of N-Cadherin, E-Cadherin, Vimentin, and α-SMA were evaluated by Western blot at 24 h **(a)**. **b)** Quantification of expression normalized to β-actin and relative to Control.

In the current reporting period, we examined the effect of Dextran-TPP coated mitochondria transplantation on epithelial (E-cadherin) and mesenchymal cell markers (N-cadherin, vimentin, and α-SMA) in A549 cells treated with TGF-β (**Fig. 7**). Dextran-TPP coated mitochondria transplantation to TGF-β-stimulated A549 cells had no effect on N-cadherin. Dextran-TPP coated mitochondria treatment resulted in an increase in the expression of E-cadherin. Moreover, Dextran-TPP coated mitochondria transplantation to TGF-β-stimulated A549 cells resulted in decreased expression of the mesenchymal proteins vimentin and α-SMA. This work corresponds to Subtask 7 in Major Task 4 of the project. These results suggest that Dextran-TPP coated mitochondria transplantation is capable of offsetting and correcting cellular dynamics that play a major role in driving IPF progression. The goals that we did not meet were examination of the effect of Dextran-TPP coated mitochondria transplantation in alveolar macrophages.

In the previous reporting period, intratracheal (IT) administration of Dextran-TPP coated mitochondria was explored in a bleomycin mouse model of pulmonary fibrosis. Epifluorescence findings showed substantial accumulation of Dextran-TPP coated mitochondria in lungs after IT administration, with minimal to no fluorescence signal detected in other major organs at different timepoints. This work was associated with Subtask 8 in Major Task 5 of the proposed work. The goal we did not meet in the current reporting period was examination of co-localization of Dextran-TPP coated mitochondria with alveolar epithelial cells, alveolar macrophages, and fibroblasts via immunofluorescence analysis (Subtask 8, Major task 5).

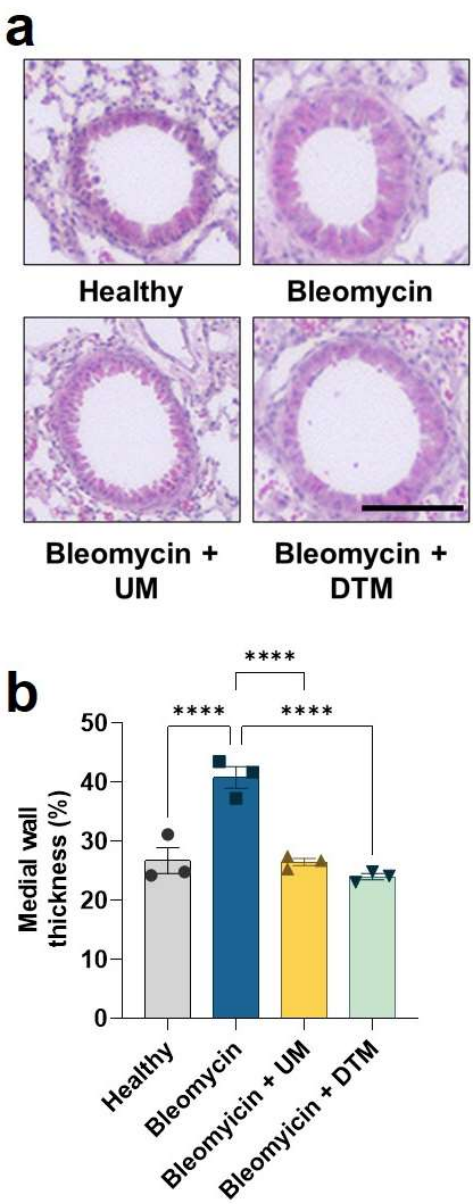

Figure 8. DTM treatment affected pulmonary vasculature in a bleomycin mouse model of IPF. Representative H&E images of tissue sections of lungs with emphasis on vascular wall thickening **(a)**. Scale bar = 100 μm. Quantification performed on pulmonary vessels (N=15) of 3 lungs/group **(b)**. Results are mean ± SEM. Two-way ANOVA followed by Tukey's multiple comparison test were used to determine statistical probabilities. ****P≤0.0001 considered as statistically significant.

In the current reporting period, we examined the efficacy of Dextran-TPP coated mitochondria in the bleomycin mouse model. In the treatment setting, mitochondria were administered IT on day 14 after model induction and weekly thereafter until the end of the experiment on day 28. This timepoint was selected based on molecular and histologic evidence of fibroblast activation, deposition of ECM, and fibrosis between days 10-21. On day 28, mice were euthanized and lung tissue collected for pathological analysis via hematoxylin and eosin (H&E). As can be seen in **Figure 8**, Dextran-TPP coated mitochondria and uncoated mitochondria (UM) had a significant effect on bleomycin-induced pulmonary vasculature, decreasing medial

hypertrophy compared to non-treated disease controls. This work comprises Subtask 9 in Major Task 6 of the proposed work. The goals we did not meet is a full characterization of the efficacy of Dextran-TPP coated mitochondria in the bleomycin mouse model. Our current work is focused on Subtasks 10-12 of Major Task 6: 1) examining the number of inflammatory cells and populations in bronchoalveolar lavage fluid (BALF); 2) examining levels of TGF-β1 in BALF, along with cytokines (e.g. IFN-γ, IL-4, IL-12, IL-13); 3) determining extent of fibrosis; 4) performing immunohistochemistry for E-cadherin and α-SMA; 5) evaluating collagen accumulation; and 6) Western blot to determine expression of fibronectin, α-SMA, E-cadherin, N-cadherin, vimentin, p-SMAD2/3, SMAD2/3, TGF-β1, HIF-1α, and type 1 collagen.

To summarize, in this reporting period we have shown that Dextran-TPP coated mitochondria transplantation can reduce the glycolytic phenotype of fibroblasts and alveolar epithelial cells, as well as prevent pro-fibrotic cellular dynamics that contribute to IPF progression. In the next reporting period, we will conclude the evaluation of our mitochondrial transplantation strategy in fibroblasts and epithelial cells and begin evaluating the strategy in alveolar macrophages. Concomitantly, we will finalize the evaluation of the efficacy of Dextran-TPP coated mitochondria treatment in the bleomycin-induced model of pulmonary fibrosis.

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

The project has provided training and contributed towards the professional development of Dr. Suhong Wu, PhD, a Research Associate, and Gherardo Baudo, MS, a graduate student working towards his PhD. The project provided opportunities for Dr. Wu and Mr. Baudo to gain knowledge and skill sets in fields that are vastly different from their training background. Dr. Wu is a polymer physicist by training with expertise in gene delivery, while Mr. Baudo received training as a mechanical engineer. The project has enabled them to obtain knowledge in the areas of cellular metabolism/metabolic pathways and mitochondrial dynamics. Dr. Wu learned new skills involving metabolic assays and became proficient in obtaining measurements of ECAR and OCR using Seahorse instruments. This project enabled Mr. Baudo to gain more experience in molecular biology techniques including cell maintenance, migration and proliferation assays, and western blots. Moreover, Mr. Baudo gained *in vivo* expertise, specifically in mouse handling, establishment of the bleomycin model in mice, and collection of BALF and lung tissues for analysis. Lastly, both Dr. Wu and Mr. Baudo have attended seminars hosted on subjects involving metabolic profiling of cells, and on chronic lung diseases such as pulmonary fibrosis and pulmonary arterial hypertension (PAH).

How were the results disseminated to communities of interest?

Nothing to Report

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

In the next reporting period, we plan to focus heavily on the following: 1) conclude all work involving Dextran-TPP mitochondria transplantation to fibroblasts and alveolar epithelial cells; 2) determine the effect of mitochondrial transplantation in alveolar macrophages; and 3) complete the efficacy evaluation of our strategy in the bleomycin model of lung fibrosis in mice.

4. IMPACT

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

Findings from this reporting period will impact the base of knowledge, theory, and research in the principal disciplinary field of the project – pulmonary fibrosis. Results show that TGF-β indeed increases glycolytic flux in fibroblasts, and that Dextran-TPP mitochondria transplantation to TGF-β-stimulated fibroblasts and alveolar epithelial cells can reduce the expression of glycolytic enzymes. Moreover, our results from this reporting period show that decreasing the glycolytic phenotype (i.e. metabolic reprogramming) of these cells through Dextran-TPP mitochondria transplantation prevented or offset cellular dynamics (e.g. EMT, fibroblast activation) that, when unmodulated, contribute immensely to the pro-fibrotic microenvironment in pulmonary fibrosis. Lastly, our preliminary results regarding the efficacy of our Dextran-TPP mitochondria transplantation in a bleomycin mouse model of pulmonary fibrosis are encouraging. Our findings show the potential for treatments in IPF that target dysregulated bioenergetics in fibroblasts and alveolar epithelial cells, specifically pharmacotherapies or interventions capable of lowering glycolysis. Importantly, our findings continue to show the potential of mitochondrial transplantation as a strategy that can impact aberrant metabolic signatures in cells involved in disease progression.

What was the impact on other disciplines?

Metabolic dysregulation and mitochondrial dysfunction is rapidly being recognized as hallmarks in a variety of diseases, including but not limited to: PAH, chronic obstructive pulmonary disease (COPD), and Alzheimer's disease. In this reporting period, we have clearly shown that our mitochondrial transplantation strategy has the potential to regulate aberrant cellular bioenergetics, preventing cellular dynamics involved in disease progression. We have shown that mitochondrial transplantation can alter cellular metabolism, resulting in a shift away from glycolysis. Moreover, we demonstrated that this shift towards favorable metabolic phenotypes abrogates processes necessary for disease progression.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to report

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

The project experienced delays. In the initial stages of the work, we struggled with ensuring viability and proper functioning of isolated mitochondria. Consequently, a delay in studies propagated throughout the funding period, resulting in later start dates for several tasks, including *in vivo* assays. These issues have all been resolved.

We also experienced a change in personnel associated with the award. Dr. Suhong Wu, the postdoctoral fellow and essential lab member on the grant, left our laboratory. Thus, Mr. Gherardo Baudo, a graduate student in the laboratory, transitioned to the role of essential lab member of the grant. The transition to new personnel resulted in an interruption and delay in the completion of the scope of the work.

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

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS

Publications, conference papers, and presentations

Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers, and presentations.

Abstract # MHSRS-20-01601 “Mitochondrial replenishment of fibroblasts and alveolar epithelial cells in idiopathic pulmonary fibrosis” was accepted for an Oral Presentation in the scientific breakout session *Combating Long-Term Respiratory Consequences of Airborne Hazards on the Battlefield* at the 2020 Military Health System Research Symposium (MHSRS).

Acceptance of Abstract # MHSRS-21-03164 “Transplantation of bioengineered mitochondria into alveolar epithelial cells and fibroblasts abrogates cellular dynamics that contribute to idiopathic pulmonary fibrosis” was accepted for a Poster Presentation in the scientific breakout session *Acute Lung Injury in Trauma and Critical Illness* at the 2021 Military Health System Research Symposium (MHSRS).

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	**Elvin Blanco**
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-7683-3311
Nearest person month worked:	1.2
Contribution to Project:	PI of the proposed work. Oversees all aspects of the work and directly supervises the research staff.

Name:	**Dale Hamilton**
Project Role:	Co-Investigator
Nearest person month worked:	0.2
Contribution to Project:	Provides insights into bioenergetic pathways and cellular metabolism, provides resources in the form of Seahorse and Oroboros instrumentation.

Name:	**Harry Karmouty-Quintana**
Project Role:	Co-Investigator
Nearest person month worked:	0.6
Contribution to Project:	Provides mechanistic insights into pathology of idiopathic pulmonary fibrosis.

Name:	**Suhong Wu**
Project Role:	Research Associate
Nearest person month worked:	.2
Contribution to Project:	Research associate assigned to the project. Performs all experiments involved in the project.

Name:	**Gherardo Baudo**
Project Role:	Graduate Research Fellow
Nearest person month worked:	4
Contribution to Project:	Performs all experiments involved in the project.

Name:	**Scott Collum**
Project Role:	Postdoctoral Fellow
Nearest person month worked:	3
Contribution to Project:	Assists Dr. Wu with molecular biology assays.

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

Yes, the support for Dr. Blanco has changed (see attached).

What other organizations were involved as partners?

University of Texas Health Science Center at Houston

8. SPECIAL REPORTING REQUIREMENTS

Not applicable

9. APPENDICES

None

BLANCO, E.

CURRENT
Title: Mitochondrial transplantation: a novel therapy for lung fibrosis
Time Commitments: 12%
Supporting Agency: DOD/PRMRP
Performance Period: 6/1/2019-11/30/2021
Brief description of project's goals: The objective of this work is to restore favorable metabolic phenotypes in lungs undergoing idiopathic pulmonary fibrosis (IPF) through mitochondrial delivery to alveolar epithelial type II cells, fibroblasts, and macrophages.
Specific Aims: 1) Evaluate the capacity of bioengineered mitochondria to restore cellular energetics in IPF fibroblasts, AE2 cells, and macrophages; 2) Determine whether bioengineered mitochondrial transplantation can treat experimental IPF.
Role: PI

Title: Mitochondrial transplantation as a strategy to metabolically reprogram macrophages in atherosclerotic lesions
Time Commitments: 5%
Supporting Agency: American Heart Association
Grants Officer: April Ciesla, April.Ciesla@heart.org
Performance Period: 7/1/2019-6/30/2021
Brief description of project's goals: The objective of this proposal is to rebalance inflammatory responses in atherosclerotic lesions by restoring favorable metabolic phenotypes in M1 macrophages.
Specific Aims: 1) Evaluate the capacity of bioengineered mitochondria to restore cellular energetics in M1 macrophages. 2) Determine whether bioengineered mitochondrial transplantation can treat experimental atherosclerosis.
Role: PI

Title: Disrupting Six/Eya signaling as new therapy for lung fibrosis
Time Commitments: 5%
Supporting Agency: DOD/PRMRP
Performance Period: 5/15/2019-11/14/2021
Brief description of project's goals: The goal of this proposal is to determine whether Six1/Eya can be targeted pharmacologically or through gene therapy approaches.
Specific Aims: 1) Evaluate whether drugs targeting the Six1/EYA complex are able to treat experimental lung fibrosis; 2) Determine the capacity of gene therapy approaches to silence the Six1/EYA axis.
Role: Co-Investigator

(New)
Title: Metabolic reinforcement of T cells for cancer immunotherapy potentiation
Time Commitments: 1%
Supporting Agency: Golfers Against Cancer
Grants Officer: Tiffany L. Polk, tlpolk@houstonmethodist.org
Performance Period: 7/27/2020-12/31/2021
Brief description of project's goals: The goal is to transplant mitochondria into T cells and deptermine if reinforcing their bioenergetic fitness makes them more capable of surviving the immunosuppressive TME, consequently bolstering T cell antitumor immunity.
Specific Aims: 1) Demonstrate that bioengineered mitochondrial transplantation into T cells improves T cell immune function in tumors. 2) Evaluate the synergy between metabolically-reinforced T cells and PD-1 inhibitors
Role: PI

COMPLETED

(Removed)
Title: Nanoparticle-mediated hyperthermia to improve chemotherapeutic efficacy in HIPEC
Time Commitments: 10%
Supporting Agency: Cancer Prevention and Research Institute of Texas
Performance Period: 8/1/2018-2/28/2021
Brief description of project's goals: The goal of this proposal is to create a nanoparticle platform that exploits hyperthermia to provide precision drug delivery to peritoneal malignancies.
Specific Aims: 1) Develop an innovative hydrogel film optimized to generate controlled, mild hyperthermia and provide local drug delivery; and 2) Determine if hyperthermia generated by a gold nanorod-embedded hydrogelchemotherapy film increases vascular permeability and chemotherapeutic efficacy
Role: co-I (8/1/18-5/31/20), PI (6/1/20-2/28/20)

(Removed)
Title: mmRNA-Based Transdifferentiation of Fibroblasts to Inducible Vascular Endothelial Cells in Myocardial Infarction
Time Commitments: 3%
Supporting Agency: George and Angelina Kostas Research Center for Cardiovascular Nanomedicine - HMRI Office of Strategic Research Initiative
Grants Officer: TBD, StrategicResearchOSRI@houstonmethodist.org
Performance Period: 11/2017-12/2020
Brief description of project's goals: The goal of this project is to examine mmRNA-based nanotherapeutics to promote fibroblast transdifferentiation to endothelial cells (MEndoT) and the potential role for cardiovascular regeneration.
Role: PI

(Removed)
Title: Re-Energizing Failing Hearts through Systemic Transplantation of Polymer-Functionalized Mitochondria
Time Commitments: 2%
Supporting Agency: George and Angelina Kostas Research Center for Cardiovascular Nanomedicine - HMRI Office of Strategic Research Initiative
Grants Officer: TBD, StrategicResearchOSRI@houstonmethodist.org
Performance Period: 11/2017-12/2020
Brief description of project's goals: The goal of the proposed work is to examine the potential of mitochondrial transplantation to increase contractility of isolated cardiomyocytes and examine the effect of systemically administering functional mitochondria to mice undergoing heart failure.
Role: PI

OVERLAP
None

PENDING
None