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PRINCIPAL INVESTIGATOR: Elvin Blanco

CONTRACTING ORGANIZATION: METHODIST HOSPITAL RESEARCH INSTITUTE
6670 BERTNER AVE
HOUSTON TX 77030-2602

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Elvin Blanco

E-Mail:eblanco@houstonmethodist.org

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6670 BERTNER AVE
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Idiopathic pulmonary fibrosis (IPF) is a progressive and irreversible disease with no effective pharmacotherapeutic treatments. 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 towards increased glycolysis, and causing mitochondrial dysfunction. In fibroblasts, TGF-β increases expression of glycolytic enzymes and glucose transporters, as well as lactate production. In alveolar epithelial 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, while in IPF alveolar epithelial 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, alveolar epithelial cells, and macrophages in IPF lungs with the goal of restoring a favorable metabolic phenotypes and mitochondrial function that can attenuate or reverse the disease. Our previous findings highlight that TGF-β treatment of fibroblasts resulted in metabolic reprogramming away from glycolysis and reduced myofibroblast-to-fibroblast transition. Herein, we show that mitochondria coated with a Dextran-triphenlyphosphonium (Dextran-TPP) polymer decreased glycolytic enzymes (HIF-1α, LDH) in fibroblasts and reduced the expression of fibroblast-to-myofibroblast differentiation and epithelial-mesenchymal transition (EMT) markers in fibroblasts and alveolar epithelial cells. Dextran-TPP mitochondria treatment also reduced proliferation and migration of TGF-β-stimulated fibroblasts and alveolar epithelial cells *in vitro*, and reduced fibrosis (collagen) in a bleomycin mouse model of IPF. 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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1. INTRODUCTION

Interstitial lung diseases like idiopathic pulmonary fibrosis (IPF) are becoming more and more prevalent among US military personnel and veterans, with an increasing trend of incidence of the disease in personnel that were exposed to dust and sandstorms, as well as industrial fires. IPF is a fatal, irreversible disease with a dismal prognosis and mortality rate. There are currently no effective pharmacotherapy-based treatments for IPF. The disease stems 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 and mitochondrial dysfunction in IPF lungs. In fibroblasts, TGF-β increases expression of glycolytic enzymes and glucose transporters, with findings showing heightened lactate production as well. In alveolar epithelial 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, while in IPF alveolar epithelial 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, alveolar epithelial 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, alveolar epithelial 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, 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, alveolar epithelial 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 42
Percentage of completion: 66%

Milestone 2: IACUC approval for *in vivo* experiments
Proposed completion date: month 3
Percentage of completion: 100% (completed 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 42
Percentage of completion: 60%

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 36
Percentage of completion: 100%

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 42
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: Site-specific accumulation and long-term persistence of polymer-coated mitochondria in IPF lungs
Proposed completion date: month 42
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
Proposed completion date: month 42
Percentage of completion: 50%

What was accomplished under these goals?

Our goal was to metabolically reprogram and restore mitochondrial function in specific cell types in IPF lungs. We hypothesized that mitochondrial delivery to fibroblasts, alveolar epithelial cells, and alveolar macrophages would properly regulate cellular bioenergetics and mitochondrial dynamics, preventing IPF progression in an experimental mouse model of the disease.

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

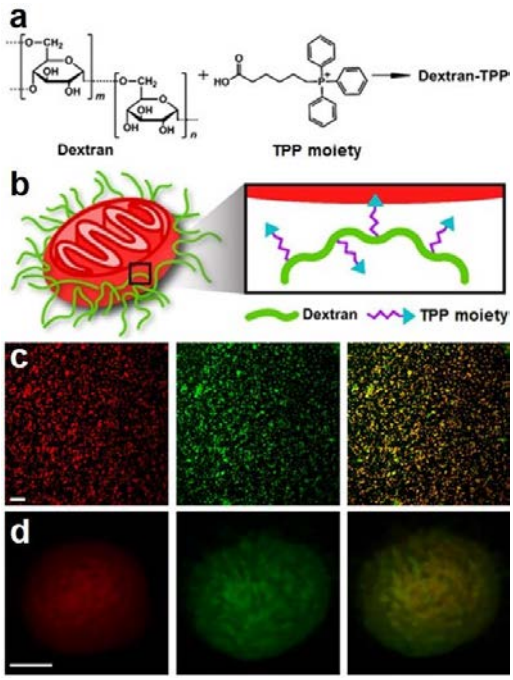

Figure 1. Dex-TPP coating of isolated mitochondria (Dex-TPP/Mt). **a**) Chemical structures of dextran and TPP. **b**) Schematic of a Dex-TPP coated mitochondrion, highlighting TPP incorporation into the mitochondrion. **c**) Confocal microscopy of Dex-TPP coated mouse liver-derived mitochondria. Dex-TPP 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 (Dex-TPP/Mt, **Fig. 1b**). The polymer conjugate was found to 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 oxidative phosphorylation and shift away from a glycolytic phenotype was observed following transplantation of Dex-TPP/Mt to cells.

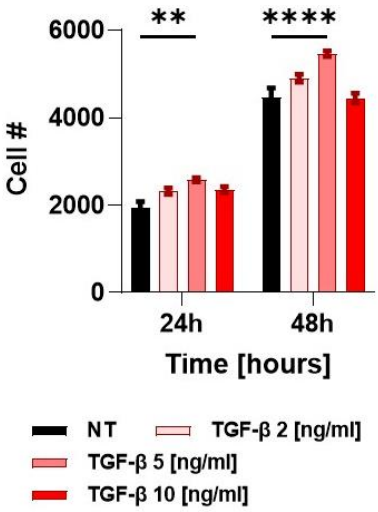

Figure 2. TGF-β leads to an increase in fibroblast proliferation. MRC-5 fibroblasts, treated with increasing doses of TGF-β, were evaluated for cell proliferation by DAPI staining and cell counting at timepoints of 24 and 48 h. ****P≤0.0001, **P≤0.01.

In previous reporting periods, our results showed that TGF-β stimulation of human lung fibroblasts (MRC-5 cells) contributed to mitochondrial dysfunction and glycolytic reprogramming. TGF-β stimulation increased glycolytic enzyme expression and resulted in an increase in the ECAR/OCR ratio – indicative of a glycolytic phenotype. Moreover, TGF-β decreased ATP production and increased glucose consumption. MRC-5 and alveolar epithelial cells (A549) stimulated with TGF-β had upregulated Smad signaling. In the last reporting period, TGF-β stimulation was shown to result in an activated fibroblast phenotype, increased expression of fibroblast-to-myofibroblast differentiation markers, and induction of epithelial-mesenchymal transition (EMT). In the current reporting period, we were able to demonstrate the effect of TGF-β stimulation on fibroblast proliferation. MRC-5 proliferation increased at 24 and 48 h timepoints with increasing concentration of TGF-β (**Fig. 2**). The only goal we have not met is to establish the conditions for TGF-β stimulation of alveolar macrophages (Subtask 1, Major Task 1).

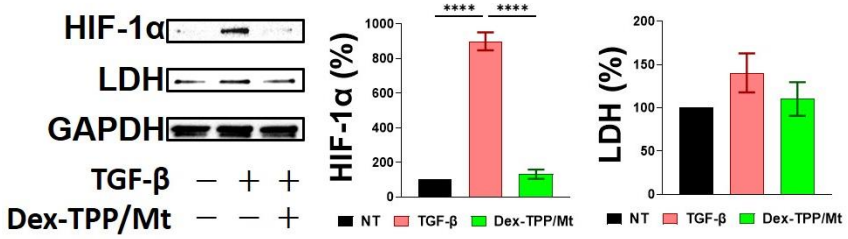

Figure 3. Dex-TPP/Mt decreased HIF-1α and LDH expression in TGF-β-treated MRC-5 cells. Expression of HIF-1α and LDH detected by Western blot and quantification of expression normalized to β-actin and relative to Control. TGF-β (5 ng/ml)-treated MRC-5 cells were incubated with 1 μg mitochondrial protein per 1.5 × 10^4 cells for 24 h. ****P≤0.0001

In previous reporting periods, uptake of Dex-TPP/Mt in MRC-5 and A549 cells was corroborated by flow cytometry and confocal microscopy. We have not yet met the goal of examining uptake and internalization of Dex-TPP/Mt in alveolar macrophages (Subtask 2, Major Task 1).

Dex-TPP/Mt treatment of fibroblasts and alveolar epithelial cells was expected to metabolically reprogram TGF-β-stimulated cells and restore mitochondrial function. In prior reporting periods, the effect of Dex-TPP/Mt treatment on TGF-β-stimulated MRC-5 cell bioenergetics was examined, highlighting a dose-dependent increase in OCR and ATP production. Bioenergetic analysis after Dex-TPP/Mt treatment of alveolar epithelial cells and macrophages remains to be

completed (Subtask 4, Major task 2). In the previous reporting period, Dex-TPP/Mt treatment of TGF-β-stimulated MRC-5 and A549 cells resulted in a decrease in the glycolytic enzymes 6-phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3 (PFKFB3) and hexokinase II (HKII) – indicative of a decrease in glycolytic flux. In the current reporting period, Dex-TPP/Mt treatment of TGF-β-stimulated MRC-5 cells resulted in decreased expression of hypoxia-inducible factor 1-alpha (HIF-1α), an important activator of glycolytic genes, and lactate dehydrogenase (LDH), an enzyme involved in the conversion of pyruvate to lactate (**Fig. 3**). Dex-TPP/Mt treatment of TGF-β-stimulated MRC-5 cells also resulted in a significant increase in ATP production and a significant decrease in glucose consumption (**Fig. 4**). Dex-TPP/Mt also had effects on mitochondrial dynamics. Dynamin-related protein 1 (DRP1) is vital to mitochondrial fission and important for maintaining mitochondrial quality control and homeostasis. Fibroblasts treated with TGF-β possess more fragmented mitochondria and increased expression of mitochondrial fission-related proteins. Dex-TPP/Mt treatment of TGF-β-stimulated MRC-5 cells resulted in a significant decrease in the expression of DRP1 (**Fig. 5**).

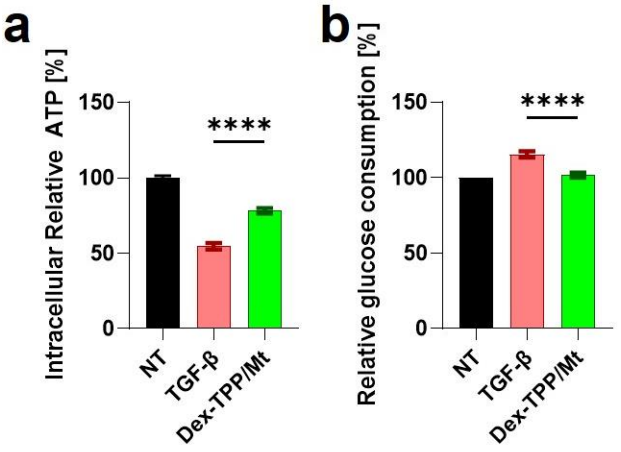

Figure 4. Dex-TPP/Mt effects on ATP production and glucose consumption. **a**) ATPLite assay of relative intracellular ATP and **b**) glucose determined via Glucose-Glo bioluminescent assay. TGF-β (5 ng/ml)-treated MRC-5 cells were incubated with 1 µg mitochondrial protein per 1.5 × 10^4 cells for 24 h. ****P≤0.0001

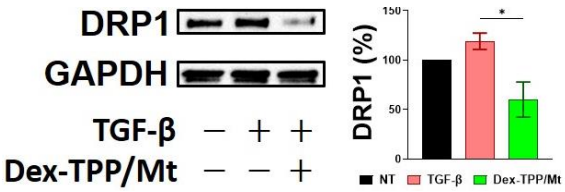

Figure 5. Dex-TPP/Mt decreased DRP1 expression in TGF-β-treated MRC-5 cells. Expression detected by Western blot and quantification of expression normalized to β-actin and relative to Control. TGF-β (5 ng/ml)-treated MRC-5 cells were incubated with 1 µg mitochondrial protein per 1.5 × 10^4 cells for 24 h. *P<0.05.

In the previous reporting period, TGF-β stimulation of MRC-5 cells was shown to phosphorylate Smad2 and Smad3, resulting in SMAD-2/3 activation and increased pro-fibrotic gene expression, including fibronectin (FN), collagens, and smooth muscle alpha-actin (α-SMA). Dex-TPP/Mt treatment of TGF-β-stimulated MRC-5 cells reduced Smad signaling and expression of fibroblast-to-myofibroblast differentiation markers compared to TGF-β-stimulated MRC-5 cells – indicative of a robust anti-fibrotic response. Previously, western blot analysis demonstrated that transplantation of Dex-TPP/Mt to TGF-β-stimulated MRC-5 cells decreased the expression of type I and IV collagens compared to TGF-β-treated MRC-5 fibroblasts. Dex-TPP/Mt treatment of TGF-β-treated MRC-5 cells also led to decreased expression of α-SMA, a protein indicative of a myofibroblast phenotype, and FN, a glycoprotein that facilitates fibroblast attachment to the extracellular matrix (ECM). In the current funding period, immunofluorescence was used to corroborate the effect that Dex-TPP/Mt had on FN and α-SMA expression in TGF-β-treated MRC-5 cells. Confocal microscopy examination confirmed reduced expression of FN and α-SMA expression after Dex-TPP/Mt treatment (**Fig. 6**).

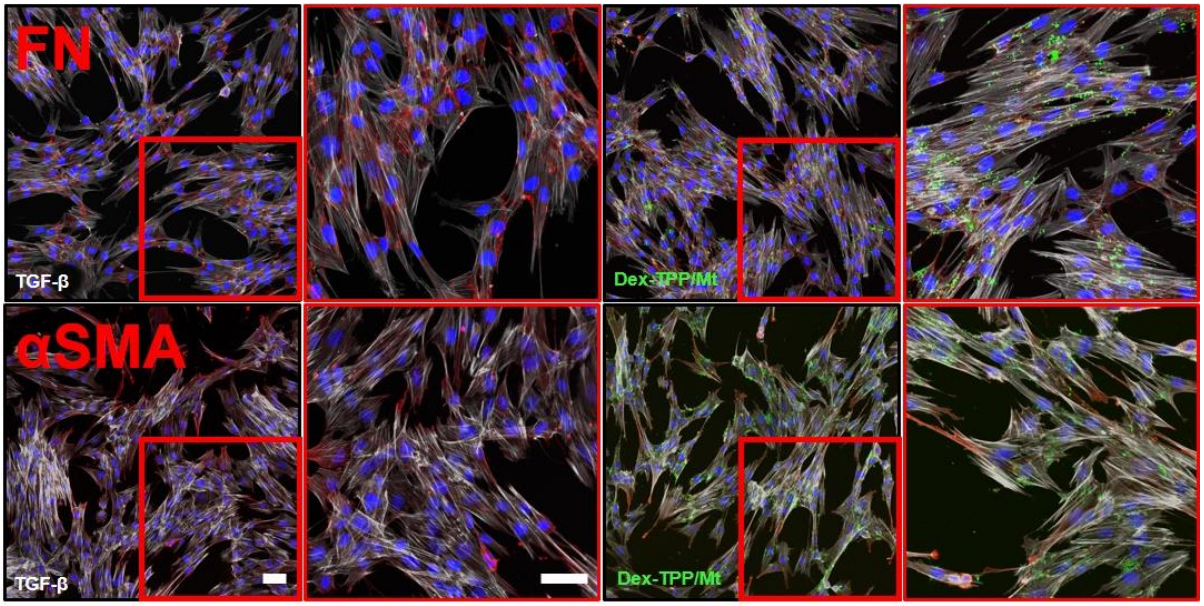

Figure 6. Immunofluorescent examination of FN and α-SMA expression in TGF-β-stimulated fibroblasts following Dex-TPP/Mt treatment. MRC-5 cells were treated with TGF-β (5 ng/ml) and Dex-TPP/Mt (1 μg mitochondrial protein per 1.5 × 10^4 cells) for 24 h and subsequently visualized via immunofluorescence with antibodies for FN and α-SMA. Blue corresponds to DAPI nuclear staining, green color corresponds to Dex-TPP/Mt, while red color is associated with FN and α-SMA in their corresponding panels. The scale bars represent 50 μm.

In the previous reporting period, the effect of Dex-TPP/Mt treatment on the glycolytic phenotype of TGF-β-stimulated alveolar epithelial cells (A549) was examined, showing a decreased expression of PFKFB3 and HKII. This shift away from a glycolytic phenotype was hypothesized to impact EMT processes. In the current period, we generated more robust findings showing SMAD-2/3 activation in A549 cells following TGF-β treatment (**Fig. 7**). Importantly, following treatment of TGF-β-stimulated A549 cells with Dex-TPP/Mt, Smad signaling decreased (**Fig. 7**).

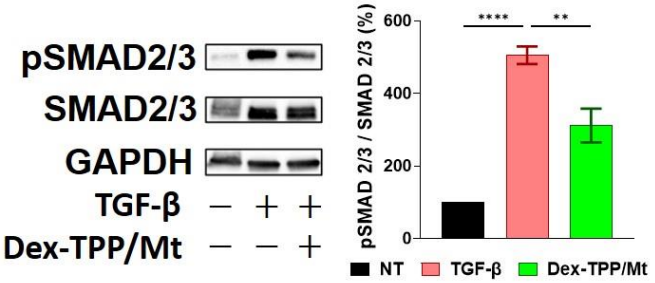

Figure 7. DTM decreased Smad signaling in TGF-β-treated A549 alveolar epithelial cells. Expression detected by Western blot at 24 h. and quantification of expression normalized to β-actin and relative to Control. TGF-β (5 ng/ml)-treated A549 cells were incubated with 1 μg mitochondrial protein per 1.5 × 10^4 cells for 24 h. ****P≤0.0001, **P≤0.01.

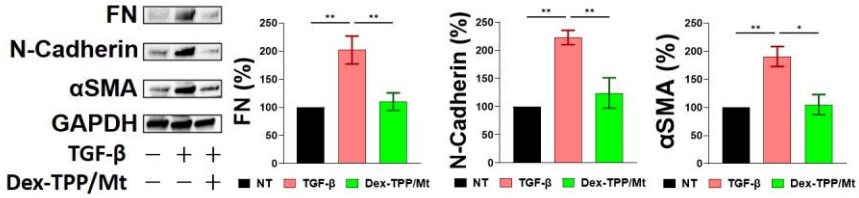

Figure 8. DTM effects on mesenchymal cell markers in TGF-β-stimulated A549 alveolar epithelial cells. Expression evaluated by Western blot at 24 h and quantification of expression normalized to β-actin and relative to Control. TGF-β (5 ng/ml)-treated A549 cells were incubated with 1 μg mitochondrial protein per 1.5 × 10^4 cells for 24 h. **P≤0.01, *P<0.05.

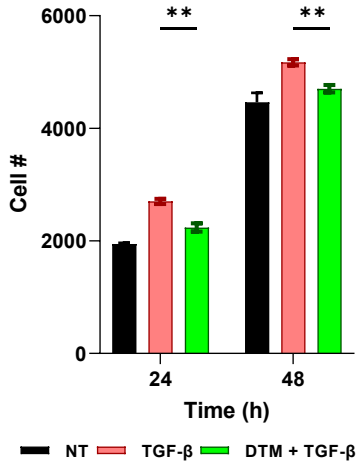

Figure 9. Dex-TPP/Mt reduced TGF-β-induced fibroblast proliferation. MRC-5 proliferation via DAPI staining and cell counting. TGF-β (5 ng/ml)-treated MRC-5 cells treated with 1 µg mitochondrial protein per 1.5 × 10^4 cells for 24 h. **P≤0.01

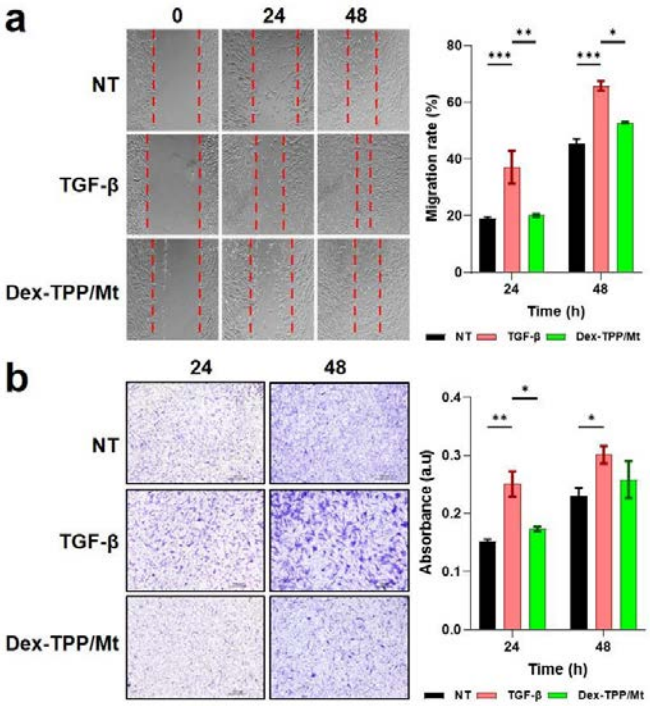

Figure 10. Dex-TPP/Mt reduced TGF-β induced fibroblast migration. MRC-5 cell migration was determined via scratch wound assay **(a)** and transwell assay **(b)**. TGF-β (5 ng/ml)-treated MRC-5 cells were incubated with 1 µg mitochondrial protein per 1.5 × 10^4 cells for 24 h. ***P≤0.001, **P≤0.01, *P<0.05.

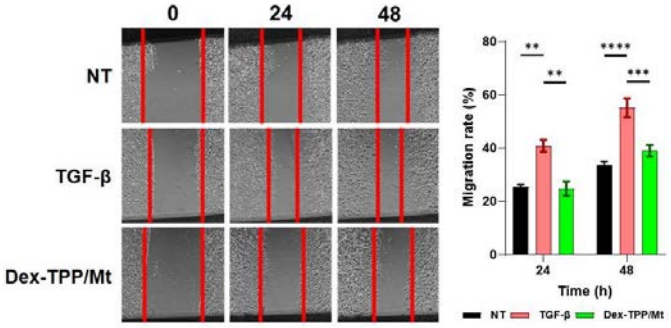

Figure 11. Dex-TPP/Mt reduced TGF-β induced alveolar epithelial cell migration. A549 cell migration determined via scratch wound assay. TGF-β (5 ng/ml)-treated A549 cells were incubated with 1 µg mitochondrial protein per 1.5 × 10^4 cells for 24 h. ****P≤0.0001, ***P≤0.001, **P≤0.01.

In the current reporting period, we also generated more robust findings regarding the effect of Dex-TPP/Mt transplantation on mesenchymal cell markers (FN, N-cadherin, and α-SMA) in A549 cells treated with TGF-β (**Fig. 8**). Dex-TPP/Mt transplantation to TGF-β-stimulated A549 cells resulted in decreased expression of mesenchymal markers.

In the current funding period, the effect of Dex-TPP/Mt on cell proliferation and migration of TGF-β-stimulated fibroblasts and alveolar epithelial cells was evaluated. Dex-TPP/Mt treatment of TGF-β-stimulated MRC-5 cells significantly reduced proliferation of TGF-β-stimulated MRC-5 fibroblasts (**Fig. 9**). Dex-TPP/Mt also reduced TGF-β-induced fibroblast migration (**Fig. 10**), with a similar effect observed in TGF-β-stimulated A549 cells (**Fig. 11**). Taken together, these results suggest that Dex-TPP/Mt 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 Dex-TPP/Mt transplantation on an alveolar macrophage pro-inflammatory phenotype.

In the previous reporting period, intratracheal (IT) administration of Dex-TPP/Mt was explored in a bleomycin mouse model of pulmonary fibrosis. Epifluorescence findings showed substantial accumulation of Dex-TPP/Mt in lungs after IT administration and the goal we did not meet in the current reporting period was examination of co-localization of Dex-TPP/Mt with alveolar epithelial cells, alveolar macrophages, and fibroblasts via immunofluorescence analysis (Subtask 8, Major task 5). In the current reporting period, we continued to examine the efficacy of Dex-TPP/Mt 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. In the previous reporting period, our findings showed that Dex-TPP/Mt had a significant effect on bleomycin-induced pulmonary vasculature, decreasing medial hypertrophy compared to disease controls. In the current funding period, we examined Masson's trichrome stained lung tissue, and found that Dex-TPP/Mt treatment of bleomycin-exposed mice reduced fibrosis (**Fig. 12**), as highlighted by the Ashcroft score, which is a semiquantitative method to score pulmonary fibrosis in bleomycin-induced models. 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 Dex-TPP/Mt 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-β in BALF, along with cytokines (e.g. IFN-γ, IL-4, IL-12, IL-13); 3) performing immunohistochemistry for E-cadherin and α-SMA; and 4) Western blot to determine expression of FN, α-SMA, E-cadherin, N-cadherin, vimentin, p-SMAD2/3, S MAD2/3, TGF-β1, HIF-1α, and type 1 collagen.

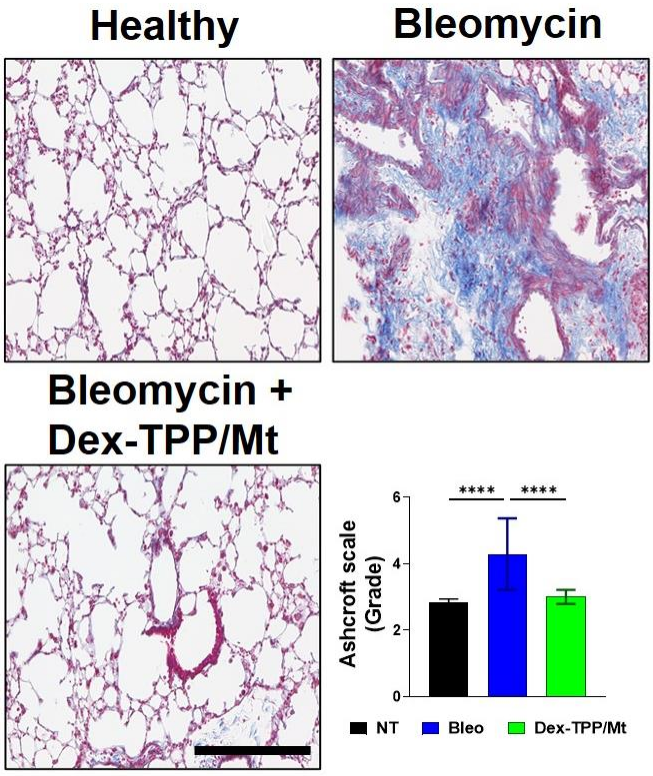

Figure 12. DTM treatment affected pulmonary fibrosis in a bleomycin mouse model of IPF. Representative Masson's trichrome images of tissue sections of lungs. Scale bar = 100 μm. Semiquantification (N=15 of 3 lungs/group) of pulmonary fibrosis performed using Ashcroft score. Results are mean ± SEM. Two-way ANOVA followed by Tukey's multiple comparison test were used to determine statistical probabilities. ****P≤0.0001 considered as statistically significant.

To summarize, in this reporting period we have shown that Dex-TPP/Mt transplantation can reduce the expression of the glyocolytic enzymes HIF-1α and LDH in TGF-β stimulated fibroblasts. Dex-TPP/Mt also led to an increase in ATP production and a reduction in glucose consumption in TGF-β stimulated fibroblasts, with a reduction in a fission-related protein

expression hinting at the potential for restoration of mitochondrial homeostasis. In the current reporting period, we include additional findings that highlight that Dex-TPP/Mt treatment of alveolar fibroblasts and epithelial cells stimulated with TGF-β can reduce myofibroblast and mesenchymal markers. Our findings show that Dex-TPP/Mt transplantation was capable of decreasing TGF-β-induced increased proliferation and migration in fibroblasts and increased migration in alveolar epithelial cells. Importantly, Dex-TPP/Mt treatment of bleomycin mice reduced pulmonary fibrosis. In the next reporting period, we will evaluate our mitochondrial transplantation 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 contributed towards the professional development of Dr. Suhong Wu and Mr. Gherardo Baudo. Dr. Wu and Mr. Baudo were able to gain knowledge and skills in new fields and disciplines. The project has enabled them to learn molecular biology and biochemistry techniques and obtain knowledge in the areas of cell metabolism and mitochondrial dynamics. Dr. Wu learned new skills involving mitochondrial isolation and characterization and Seahorse metabolic assays, becoming proficient in measurements of ECAR and OCR. 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. Dr. Wu and Mr. Baudo have attended seminars hosted on subjects involving metabolic profiling of cells and on pulmonary fibrosis.

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?

We plan to: 1) determine the effect of mitochondrial transplantation in TGF-β-stimulated alveolar macrophages; and 2) complete the *in vivo* efficacy evaluation of our strategy in mice.

4. IMPACT

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

Findings will impact the base of knowledge, theory, and research in pulmonary fibrosis. Results show that TGF-β impacts cell metabolism by increasing glycolytic enzyme expression and affecting mitochondrial dynamics. Dex-TPP/Mt treatment of TGF-β-stimulated fibroblasts and alveolar epithelial cells can offset cellular dynamics, principally fibroblast-to-myofibroblast differentiation and epithelial-to-mesenchymal transition – two processes fundamental for progression and sustainment of a pro-fibrotic microenvironment. Lastly, Dex-TPP/Mt transplantation in an experimental model of IPF showed reduced fibrosis. Our results point towards the therapeutic potential of strategies that regulate bioenergetics and mitochondrial dynamics in fibroblasts and alveolar epithelial cells in IPF. Our findings show the impact that restoration of mitochondrial function in IPF can have on disease progression, and the potential of

mitochondrial transplantation as a therapeutic strategy. It also opens several avenues for the exploration of pharmacotherapies and/or gene therapies aimed at correcting aberrant mitochondrial dynamics in IPF as viable treatment strategies.

What was the impact on other disciplines?

Mitochondrial dysfunction has been recognized as a hallmark in a variety of diseases, including PAH, chronic obstructive pulmonary disease (COPD), and Alzheimer's disease. In this reporting period, we have demonstrated that mitochondrial transplantation can prevent cellular dynamics vital to IPF progression. We have shown that mitochondrial transplantation can alter cell metabolism and regulate mitochondrial dynamics – making this strategy highly applicable as a therapy in other disease conditions whose hallmarks include mitochondrial dysfunction, as well as in those where fibrosis is also a pathological hallmark.

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

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

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).

Acceptance of Abstract # MHSRS-22-06669 “Mitochondrial Replenishment of Alveolar Epithelial Cells and Fibroblasts Alters Cell Bioenergetics and Ameliorates Pulmonary Fibrotic Mechanisms” was accepted for a Poster Presentation in the scientific breakout session *What's New in Airway Science and Technology* at the 2022 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:	0.05
Contribution to Project:	Provides insights into bioenergetic pathways and cellular metabolism.

Name:	**Gherardo Baudo**
Project Role:	Graduate Research Fellow
Nearest person month worked:	3.7
Contribution to Project:	Research associate assigned to the project. Performs all experiments involved in the project.

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. Please see attached support for Dr. Blanco.

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: 6/1/2019-11/30/2022
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: Cierra Vaugh
Performance Period: 7/1/2019-6/30/2022
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: Metabolic reinforcement of T cells for cancer immunotherapy potentiation
Time Commitments: 1%
Supporting Agency: Golfers Against Cancer
Grants Officer: Tiffany L. Polk
Performance Period: 7/27/2020-12/31/2022
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

(New)
Title: Bioenergetic potentiation in Alzheimer's Disease as a therapeutic strategy
Time Commitments: 5%
Supporting Agency: Alzheimer's Association
Grants Officer: Veronica Chavez
Performance Period: 2/1/2022-1/31/2025
Brief description of project's goals: Our goal is to deliver healthy, Dextran-TPP coated mitochondria to neuronal cells in hopes of preventing AD progression.
Specific Aims: 1) Evaluate whether bioengineered mitochondria can restore favorable energetics in metabolically compromised neurons. 2) Determine whether bioengineered mitochondria can protect against Aβ toxicity
Role: PI

COMPLETED

(Removed)

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

OVERLAP

None