Analysis of fibrotic lung cells to changes in environmental calcium and insulin

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Introduction

Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease characterized by irreversible scarring of lung tissues which ultimately leads to organ malfunction, disrupted gas exchange, and respiratory failure [1]. Patients suffering from IPF have poor prognoses, as life expectancy is typically 3 to 5 years after diagnosis. Although new medicines (i.e nintendanib and pirfendione) have been shown to delay disease progression and have received FDA approval, no treatments have been found to cure IPF. As such, most treatments focus on alleviating patient symptoms. In order for a cure to be developed, research must be conducted to characterize the etiology of this disease. Thus IPF progression was examined using laboratory maintained human lung cell lines via the analysis of cellular response to changes in extracellular calcium ion (Ca^{2+}) concentration, extracellular insulin concentration.

Methods

1. Cell culture

Human lung fibroblasts (cell line MRC-5, CCL-171™) from the American Type Culture Collection (ATTC®) were maintained in ScienCell™ complete fibroblast media (FM) in 75 cm^2 tissue culture flasks under standard conditions (37 ˚C with 5% CO_2 concentration). Cells were grown to approximately 70% confluence (Fig. 1) prior to passages to new flasks/wells and/or treatments for further assays.

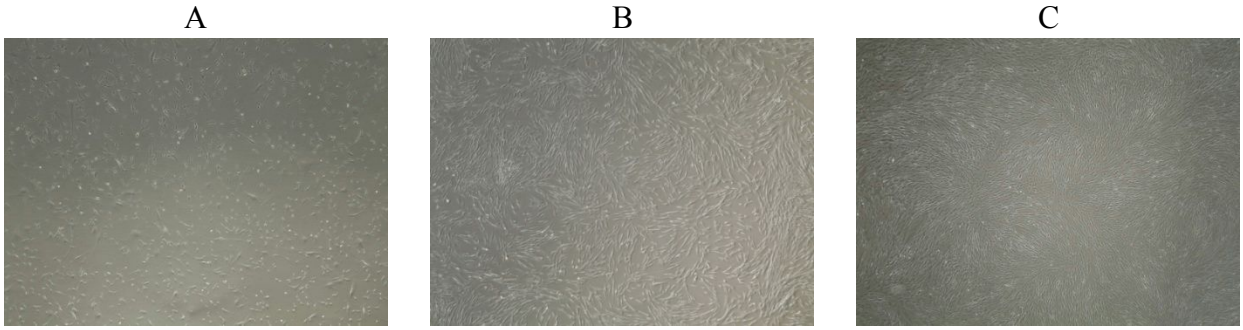


Fig. 1 MRC-5 confluence. Cells were imaged using an *EVOS* xl light microscope at 4x zoom. Images A, B, and C are approximately 10, 70, and 100 % confluent respectively.

2. Scratch Assays

Cells above 70 % confluence in 75 cm^2 culture flasks were washed with Dulbecco's phosphate buffered saline (DPBS) prior to trypsininzation with TRYP LE for 1 to 3 minutes at 37 ˚C. FM was added and the mixture was gently agitated via pipetting to ensure even distribution of cells and limit cell coagulation. The cell-containing FM was distributed in 2 mL aliquots to each well of a six well culture plate, and incubated overnight under standard conditions. Each well was then scratched in a cross pattern using a 200 µL pipette tip to simulate a "wound" and washed with DPBS. Cells were grown in serum-free media with various concentrations of insulin, ethylene glycol tetraacetic acid (EGTA) and $CaCl_2$ (Table 1). Cells were imaged at 24 hour intervals to measure change in the "wound" area using *Image J* software.

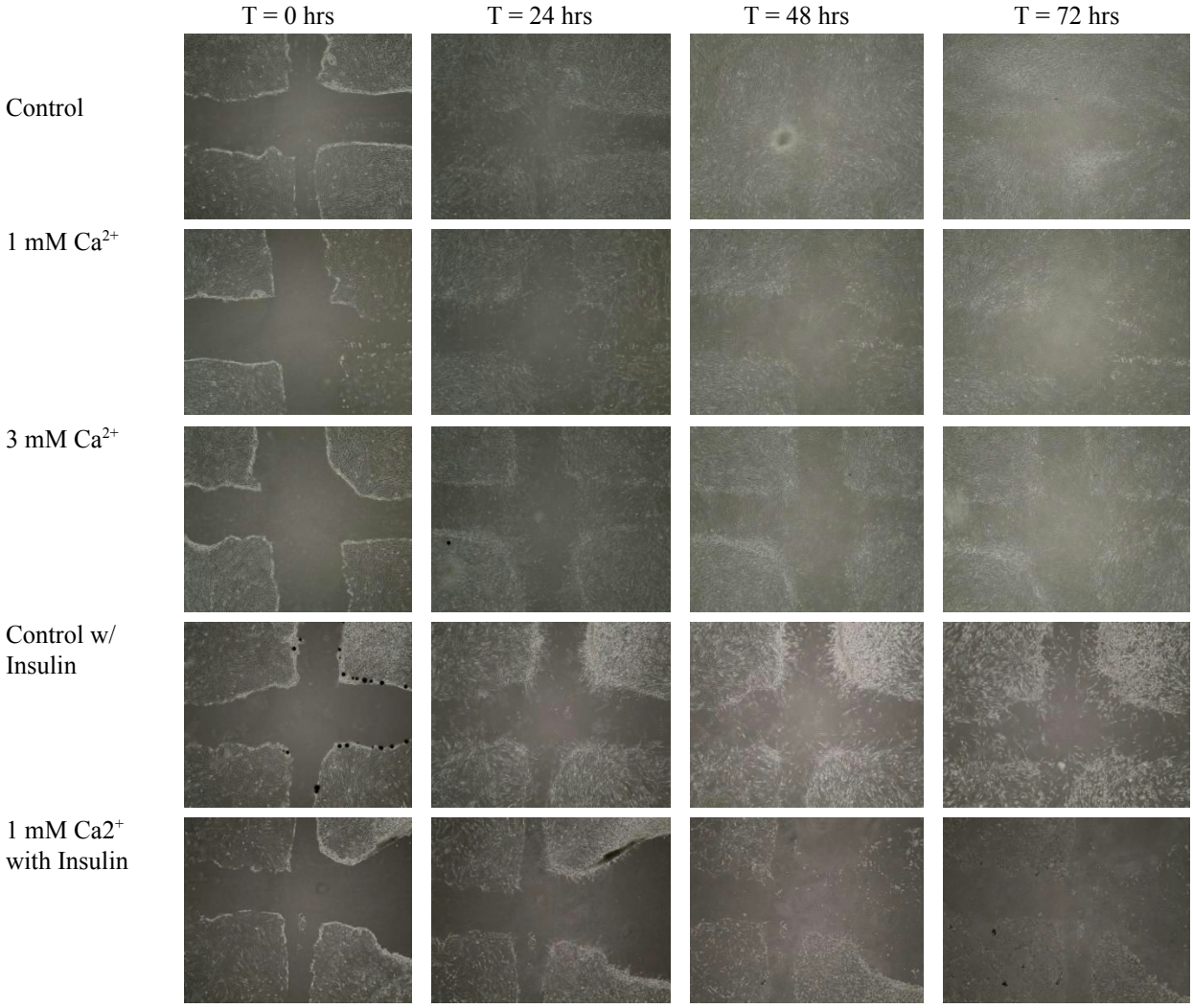
Table 1. MRC-5 Treatments in serum-free media

SAMPLE	$CaCl_2$ conc. (mM)	Insulin $3*10^{-7}$ M
1	0	no
2	1	no
3	3	no

4	0	yes
5	1	yes
6	3	yes
SAMPLE	**EGTA conc. (mM)**	**Insulin $3*10^{-7}$ M**
7	0	no
8	1	no
9	3	no
10	0	yes
11	1	yes
12	3	yes

Results

Scratch Assays



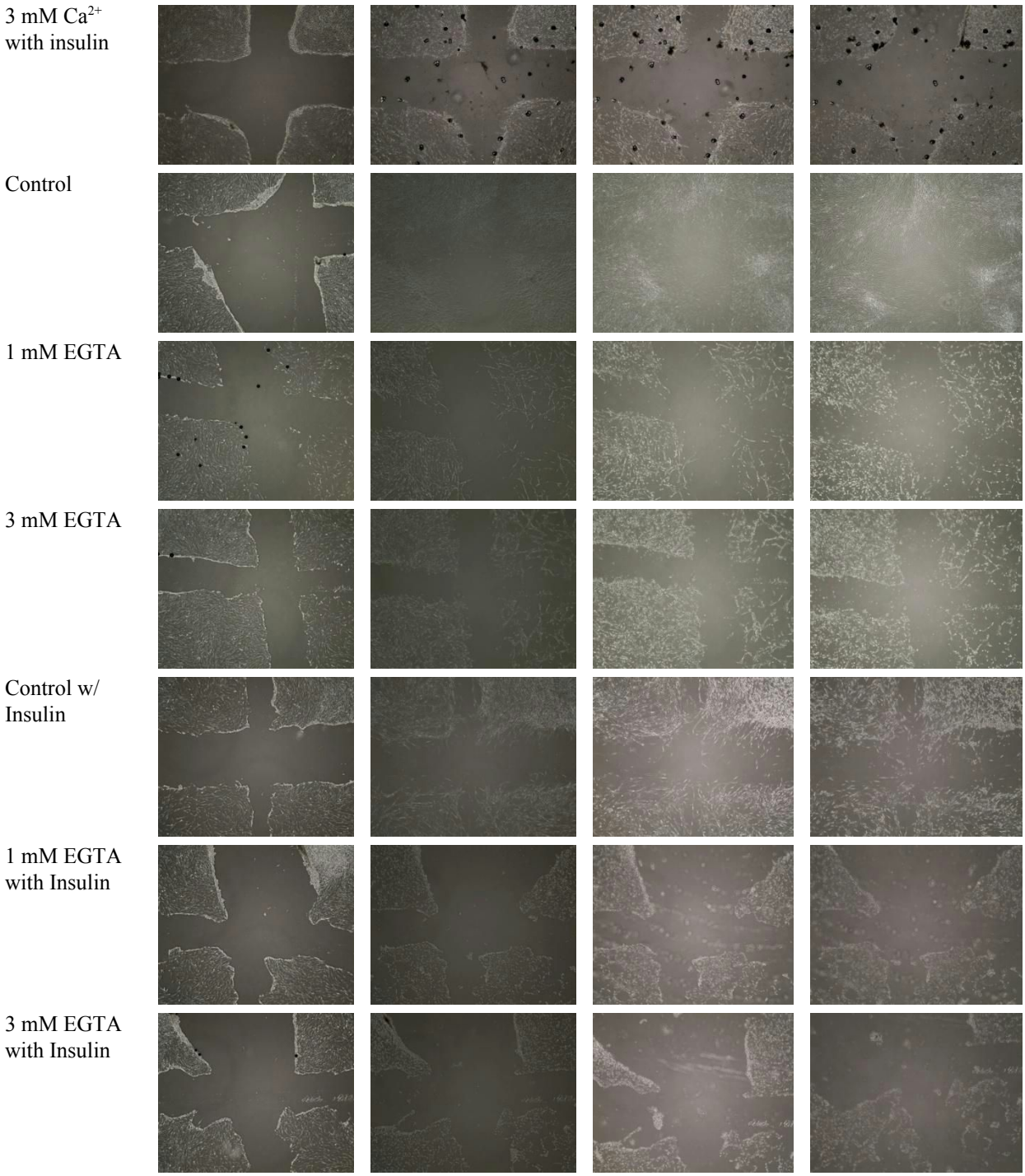


Fig. 2 Scratch Assay Images over 72 hrs. Cells were imaged at 24 hr. intervals upon infliction of "wound" with 200 µL pipette tip.

Discussion

In the scratch assays, no discernible difference in cell migration and healing between samples treated with insulin versus those without insulin. Wound healing was slightly greater in samples treated with 1 mM Ca^{2+} over the controls. However, an increase to 3 mM Ca^{2+} concentration appeared detrimental to cell migration. For samples treated with EGTA, 1 mM concentrations impeded wound healing. Treatment with 3 mM EGTA was detrimental, resulting in cell death.

Future work is needed to determine why this is so. Analysis of cell protein profiles as well as extracellular protein secretions via Western blots would aid in understanding the

molecular effects of the treatments performed. Additionally, tests using a broader range of Ca^{2+} and EGTA concentrations in more gradual increments would need to be performed given that the results suggest a concentration threshold at which treatments with either Ca^{2+} or EGTA are detrimental to cell migration.

Reflection

For this project, I served as a research assistant for Dr. Seballos' biochemistry lab of the University of the South. As such, I was tasked with researching and conducting laboratory experiments and analyzing data to better understand the etiology of idiopathic pulmonary fibrosis (IPF).

Throughout the duration of this internship, I familiarized myself with laboratory techniques such as tissue culture, protein extraction and immunoblotting. These skills not only contributed to data acquisition for the lung cell project, but also imparted upon me a greater breadth of understanding for such skills are crucial to augmenting the scientific community with additional knowledge.

In addition, I learned to collaborate with my partners. Our discussions resulted in ideas and new insights on how to address issues and answer questions in our experiments. Moreover, I became close friends with my colleagues. Our relations were highlighted with mutual sincerity and good rapport. Encouragement and support were seldom lacking regardless of experimental success or failure.

Overall, I gleaned a greater appreciation for research and its contributions to science. As a pre-med student, I find it humbling to understand how scientific knowledge – though it may seem mundane and taken for granted in a textbook – came about as result of the efforts of dedicated researchers. I hope to retain this mindset as I pursue my future academic endeavors both on and off the mountain.