Effects of Pirfenidone on Fibroblast Proliferation and Gene Expression

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INTRODUCTION

- The process of tissue healing involves blood and its various clotting factors, inflammation involving various cytokines and cell signaling markers, and the excessive production of a new extracellular matrix scaffold that often results in tissue fibrosis.
- The resulting fibrosis and/or stiffness may be debilitating and result in significant loss of joint function.
- As this complex mechanism of healing is further characterized, potential interventions into the process and therapeutic avenues to reduce the exaggerated healing response can limit tissue scarring and fibrosis.
- To mitigate collagen overproduction, excess inflammation, and excess cell proliferation at the site of injury, Pirfenidone, a drug that is currently approved for lung fibrosis, may be used to prevent this undesirable outcome in musculoskeletal applications (1).

OBJECTIVES

- Evaluate the effectiveness of Pirfenidone on mitigating fibrotic change *in vitro.*
- Provide evidence to surgeons to help prevent iatrogenic scarring and postoperative complications.
- Provide evidence for further research on a mouse model of skin and soft tissue injury and repair.

MATERIALS & METHODS

- 3T3 mouse embryonic fibroblasts were used in three different preparations and at two time points.
- The first preparation was the control group, which was cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum.
- The second preparation was the 3T3 cells with the addition of TGF-ß, which was used to induce fibroblast proliferation and collagen production to mimic tissue fibrosis. The third included both TGF-ß and pirfenidone.
- All preparations were cultured for 12 and 24 hours. After collection, analysis was done via qPCR to quantify levels of gene expression and immunohistochemistry to visualize the fibrotic changes present in the different preparation groups.
- α-Smooth muscle actin (SMA), Col1, Col3, Col6, fibrinogen, and Ki-67 were used as markers for the q-PCR.

RESULTS

- Cell gene expression for α -SMA, Col1, and Col3 drastically increased significantly in the 3T3 cells that were given TGF-ß and incubated for 24 hours (Figure 1).
- Conversely, the culture of cells that were incubated with both TGF-ß and Pirfenidone showed a marked decrease in the expression of those same biomarkers.
- Although not as drastic, levels of col6, fibrinogen, and Ki-67 also showed a slight increase in the cells given TGF-ß for 24hrs as well as a decrease in the levels of these biomarkers in the pirfenidone treated group. No noteworthy changes were observed in the 12-hour timepoint groups.
- Results for immunohistochemistry staining are not yet available but have been fixed with the layout in Figure 2.

Figure 1.

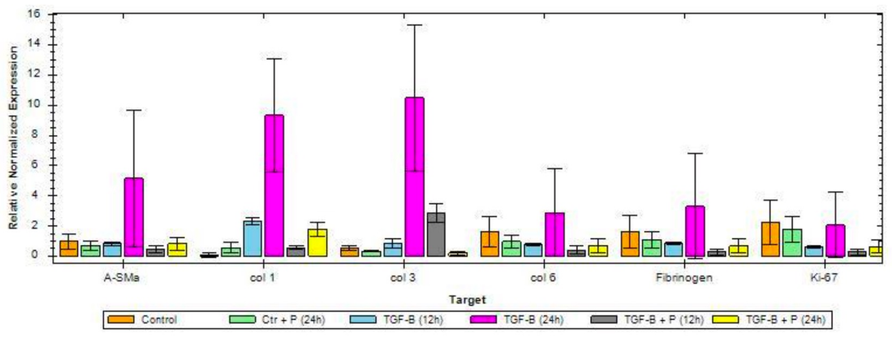
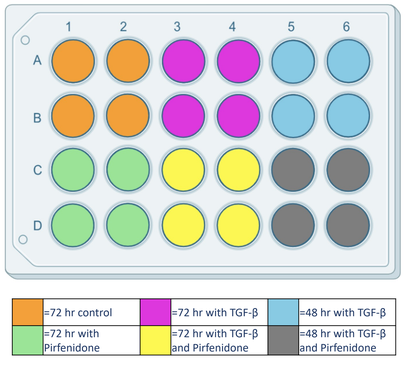


Figure 2.



CONCLUSIONS

- Based on the results we have thus far, it is suspected that the hypothesized effect of pirfenidone is true.
- α -SMA, a marker for myofibroblast phenotypic switch, is an indicator of soft tissue fibrosis. With the marked decrease in the expression of this biomarker, Pirfenidone proves to be a potent potential agent that may be able to suppress scarring in an *in vivo* model.
- The decrease in expression of all the different types of collagens as well as Ki-67, a cell proliferation marker, further proves that this drug may positively affect the excess matrix production and cell proliferation, which is the crux of soft tissue fibrosis and scarring.
- Also, based on the success of this qPCR, we hope to transition to an in vivo model to assess Pirfenidone's effect on post-operative skin, soft tissue, and joint fibrosis.

REFERENCES

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