The role of matrix stiffness in the sustained fibrotic behavior of myofibroblasts

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AIM AND OBJECTIVES

Hypothesis:
Mechanical preconditioning fibroblasts on pathological levels of stiffness permanently activates them towards the highly synthetic, contractile myofibroblast phenotype.

Objective I.
Develop a system to continuously culture lung fibroblasts within the range of stiffness seen in normal and fibrotic lung tissue.

Objective II.
Test if myofibroblasts have 'mechanical memory'.

INTRODUCTION

Two quintessential features found in all connective tissue fibrosis are 1) the activation of myofibroblasts (MFs) and 2) the replacement of normal tissue with stiff, collagen-dense matrix. In this study we evaluate whether preconditioning lung fibroblasts (LFs) on various levels of stiffness within the elasticity range of normal and fibrotic lung tissue will either protect or permanently activate LFs towards the highly synthetic, contractile MF phenotype. Fibrotic activity was determined by assaying for cell proliferation (cell counts/Ki67), α-SMA levels, and ECM production.

RESULTS SUMMARY

Establishing a new culture system

- As seen previously, expression of α-SMA and proliferation of lung fibioblasts increased with increasing substrate stiffness (5kPa versus 100 kPa).
- Myofibroblasts proliferative capacity increased with increasing stiffness.

Mechanical priming studies

- Lung fibroblasts preconditioned on soft surfaces ("P_L") demonstrated reduced levels of α-SMA expression and cell proliferation when compared with cells continuously cultured on stiff surfaces .
- Lung fibroblasts mechanically primed on stiff surfaces ("P_H") maintained the highest proliferation rates of any group, and demonstrated sustained high α-SMA levels (myofibroblast persistence).

CONCLUSIONS

Our preliminary results suggest that mechano-sensed information relating to physical conditions of the local cellular environment could provide instructions to direct or protect against fundamental long-term fibrotic behavior of fibroblasts.

ACKNOWLEDGEMENTS

This work was supported by the following sources:

BACKGROUND: Myofibroblast regulation is dependent on substrate stiffness

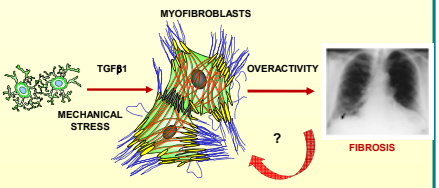

Figure 1. It has long been established that the ECM matrix regulates LF to MF differentiation. Here, we investigate the role of matrix stiffness in MF persistence.

Methods 1: Development of cell culture system

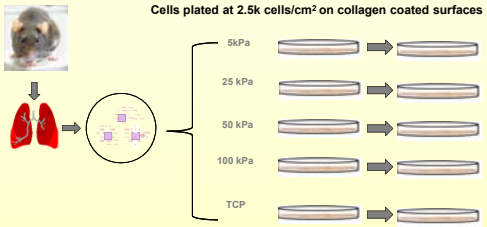

Figure 2. Rat lung explants were isolated and subsequent lung fibroblast (LF) populations were cultured on soft silicone rubber surfaces to model various stages of lung health (healthy and increasing stages of fibrosis).

Results 1: Stiffness-dependent cell outgrowth

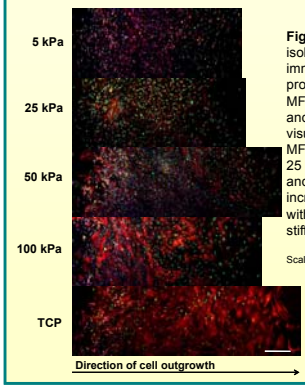

Figure 3. 6 days after isolation, cells were immunostained for proliferation (Ki67), MF presence (α-SMA), and with DAPI to visualize cell nuclei. MFs first appear on 25 kPa substrates, and subsequently increase in numbers with increasing stiffness.

Scale bar = 200 μm

Results 2: Stiffness regulates MF proliferative behaviour

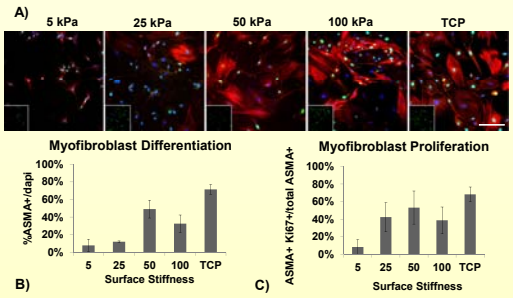

Figure 4. Passage 1 LFs were cultured for 5 days on PDMS surfaces and A) immuno-stained for α-SMA, Ki67, and DAPI. B) MF cell percentage and C) proliferative capacity increases (generally) with increasing stiffness.

Data represent combined results of two experiments (minimum of 200 cells per treatment). Scale bar = 100 μm

Results 3: Establishing stiffness-dependent cell behaviour in the PDMS culture system

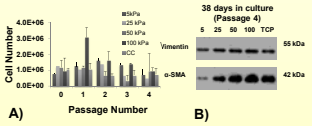

Figure 5. A) Total cell number over four passages and B) α-SMA normalized to vimentin after four passages. Although α-SMA consistently increased with increasing stiffness, cell numbers did not.

Methods 2: Characterizing 'mechanical priming'

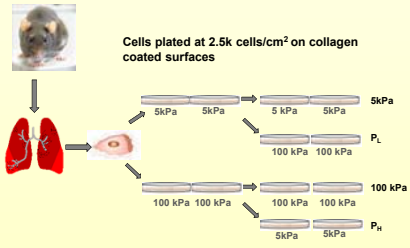

Figure 6. Rat LFs were seeded at 2.5*10⁶ cells/cm² onto collagen-coated silicone substrates and cultured for four passages to model:

1. Healthy lung tissue (5 kPa)
2. Mature fibrotic tissue (100 kPa)
3. The migration of cells from soft to fibrotic-stiff zones (P_L)
4. The migration of cells out of stiff fibrotic-zones to soft tissue (P_H)

Results 4: Sustained response to mechanical conditioning

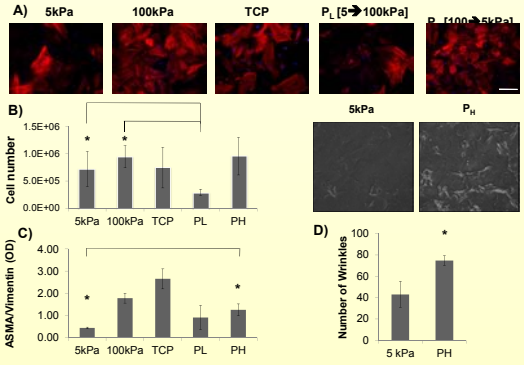

Figure 7: LFs were assessed for MF presence, proliferation, and contractility by A) immunostaining for α-SMA and DAPI, B) measuring the total cell number, C) assaying α-SMA via western blot, and D) determining wrinkling capacity 14 days after priming. Cells preconditioned on soft substrates had statistically lower cell numbers than cells cultured in 'healthy' or 'fibrotic' conditions, yet cells preconditioned on stiff surfaces maintained the 'fibrotic' phenotype even after the removal of the stiffness stimulus.

Data represent the combined results of two experiments (ps0.05). Scale bar = 100 μm

Results 5: Evaluation of mechanical priming events over time

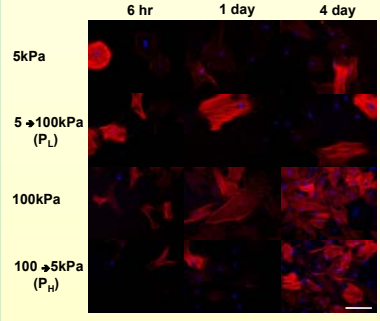

Figure 8. Time course evaluation of priming events. Although the P_L and P_H groups initially demonstrated responses to a changing stiffness as predicted by literature (6 hr), behaviours due to preconditioning were re-established after 4 days in culture.

Scale bar = 100 μm