# Mesenchymal Stem Cell Mechanical Memory at Cytoplasm and Nucleus

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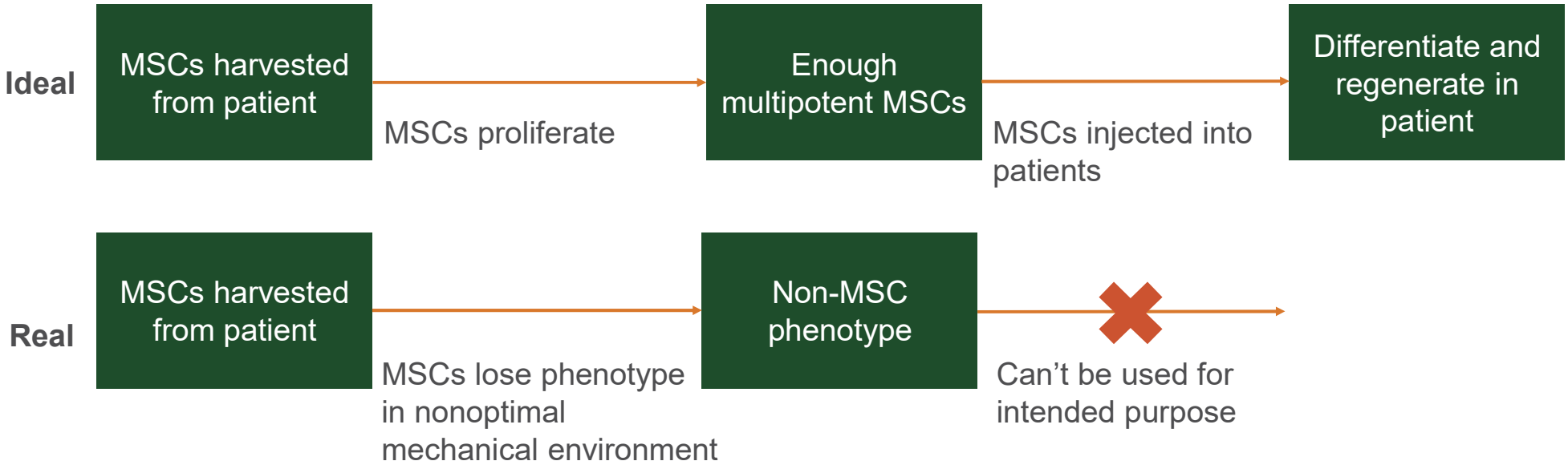
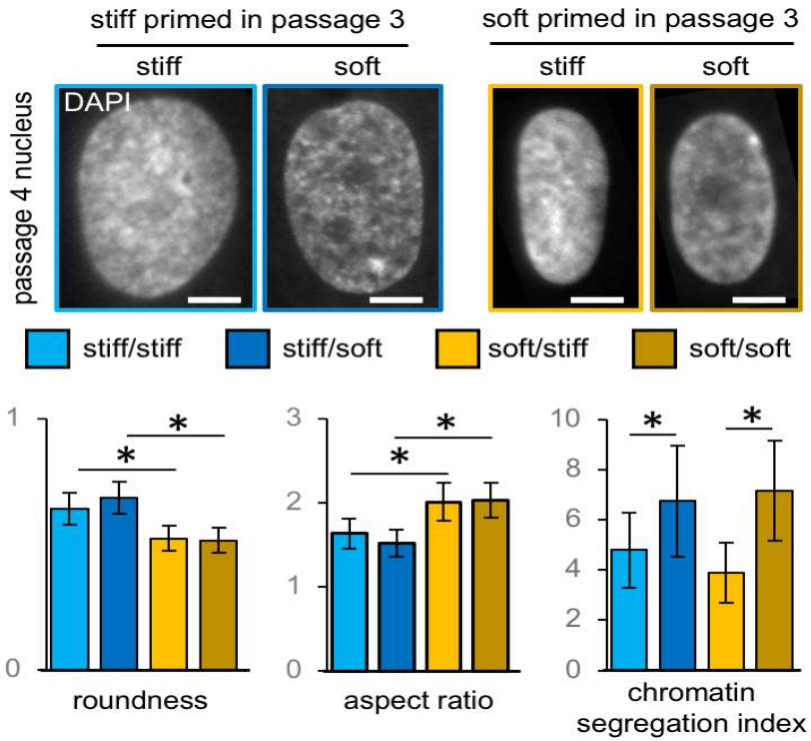
# Background

Mesenchymal stem cells (MSCs) offer a promising new frontier in regenerative and anti-aging medicine.[1-2] However, large-scale expansion of MSCs causes them to lose their multipotency.[3,4]




MSCs have **mechanical memory.** They can sometimes define their own phenotype during expansion on substrates with various stiffnesses.[3,5-8] This mechanical memory is likely regulated by structures in the cell.

# Objectives



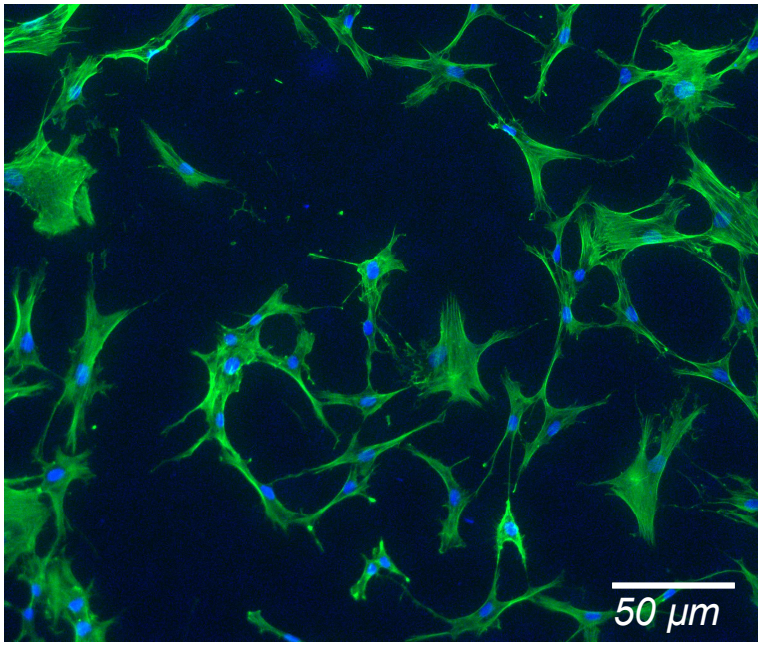

MSCs grown on stiff substrate and transferred to soft recovered desired chromatin architecture, but not nuclear shape[9]

- Explain why chromatin architecture of MSCs grown on stiff substrate recovered while the cytoplasmic structure did not, as apparent from nuclear shape
- Characterize mechanical memory of lamin (associated with nucleus) and actin (associated with cytoplasm) in MSCs
- Collect and analyze data on dynamics of lamin and actin formation and degradation
- Create a computational model to predict the dynamics of these proteins
- Explain and predict MSC plasticity and mechanical memory, removing proliferation related limitation in MSC bioengineering

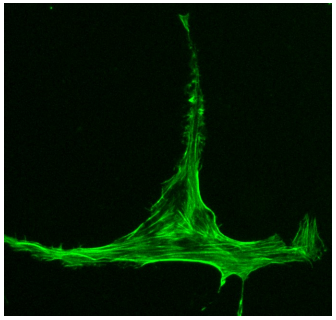
# Methods

Investigating role of actin and lamin proteins in mechanical memory through MSC culture on different substrate stiffness, confocal microscopy, image analysis and mathematical modeling:

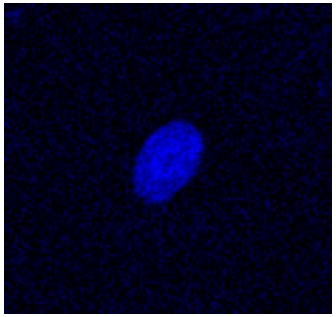
1




2a



b



Confocal microscope fluorescent images 1. MSCs cultured on soft substrate 2a. Phalloidin stained actin 2b. DAPI stained nucleus

Equations for Lamin Turnover [10,11]

$$\frac{dl}{dt} = aL - bl$$

$$\frac{dL}{dt} = cl - dL$$

*l:* mRNA for lamin production

*L:* lamin protein

*a*, *b*, *c*, *d:* kinetics constants

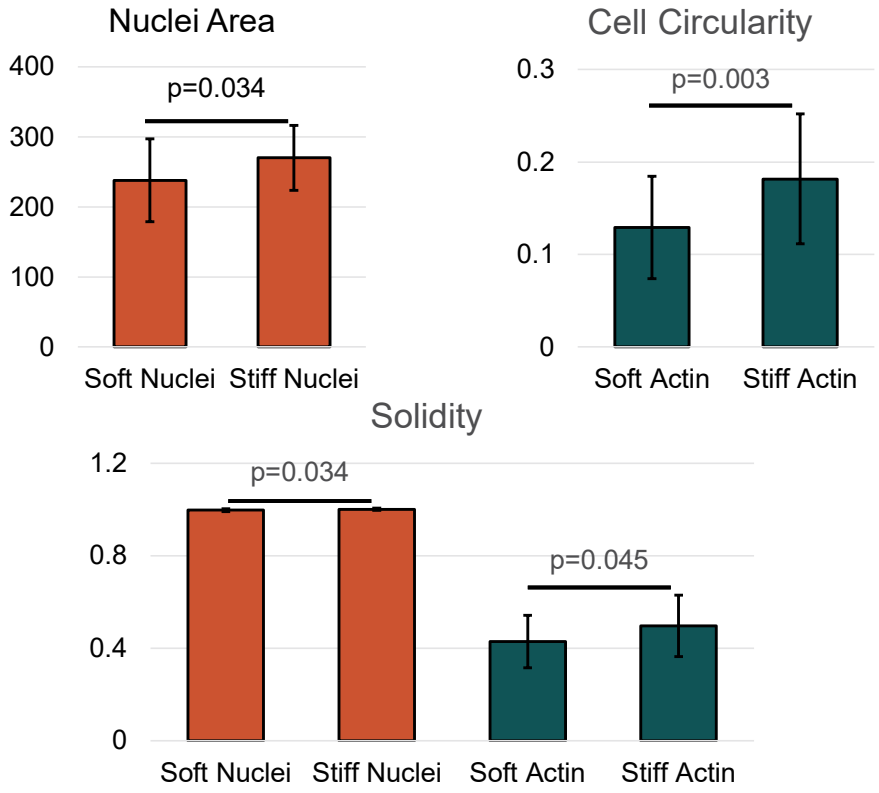
$$d = k\frac{L^{n-1}}{K_m^n + L^n}$$

$$K_m \approx Force\ on\ nucleus^{0.3}$$

$$Force\ on\ nucleus \approx L^{2.5}A^{0.5}$$

$$\frac{dL}{dt} = l - \frac{L^2}{L^{1.5}A^{0.3} + L^2}$$

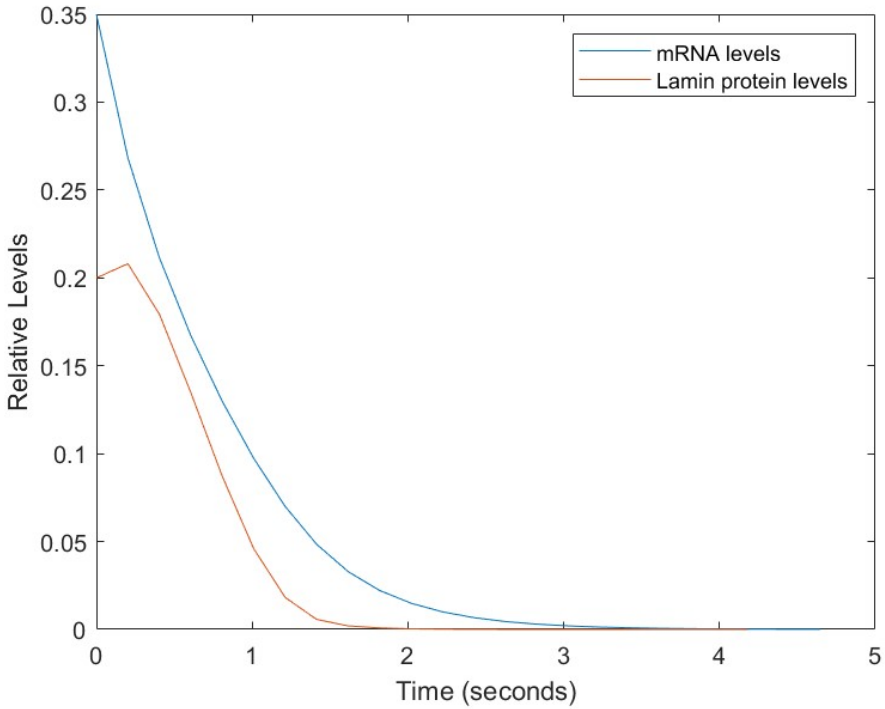
# Results




Significance: two-tailed heteroscedastic T test, $\alpha$=0.05, n > 15

Cell and nuclear shape are significantly different on stiff substrate compared to soft substrate

Levels of lamin mRNA and protein over time (MATLAB model)




Lamin expression changes based on the model, but needs further refinement

# Conclusions

From significance tests:

- Nuclei of MSCs cultured on stiff substrate are larger on average.
- Average cell circularity is higher for MSCs cultured on stiff substrate.
- Both the average actin stress fibers and DNA intensity for cells grown on stiff substrate are higher.

Overall:

- MSCs grown on stiff substrate grow more circular and spread larger. This matches observations in the literature.
- MSCs grown on stiff substrate have higher solidity in their actin footprints, indicating more stress fiber formation.
- MSCs grown on stiff substrate have higher nuclei solidity, indicating less chromatin segregation. This is also consistent with previously observed results (see Objectives).
- Lamin levels in cells exposed to stress return to a baseline biological level.

# Future Directions

- Gather more data on MSCs cultured on soft and stiff substrates
- Develop a model for polymerization and degradation of actin over time
- Refine the lamin turnover model
- Compare respective timescales of MSC lamin and actin recovery from tension
- Apply mechanical memory of MSCs to promote desired phenotype in mass production

## Benefits from SURE

The SURE experience allowed me to work hands-on in an exciting and rapidly developing field. I applied, reinforced, and expanded concepts from my coursework. Moreover, I gained specialized knowledge and practical skills in cell biomechanics and research. Finally, I gained confidence in processing, analyzing, and presenting scientific data.

## Acknowledgements

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# Thank you

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