Altered Mechanosensitivity with Modulation of Nuclear Mechanics in Fibrochondrogenic Mesenchymal Stem Cells

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Background

- **Musculoskeletal soft tissue repair**

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- **Musculoskeletal soft tissue repair**
- **Tendons/ligaments**
  - **Aligned**

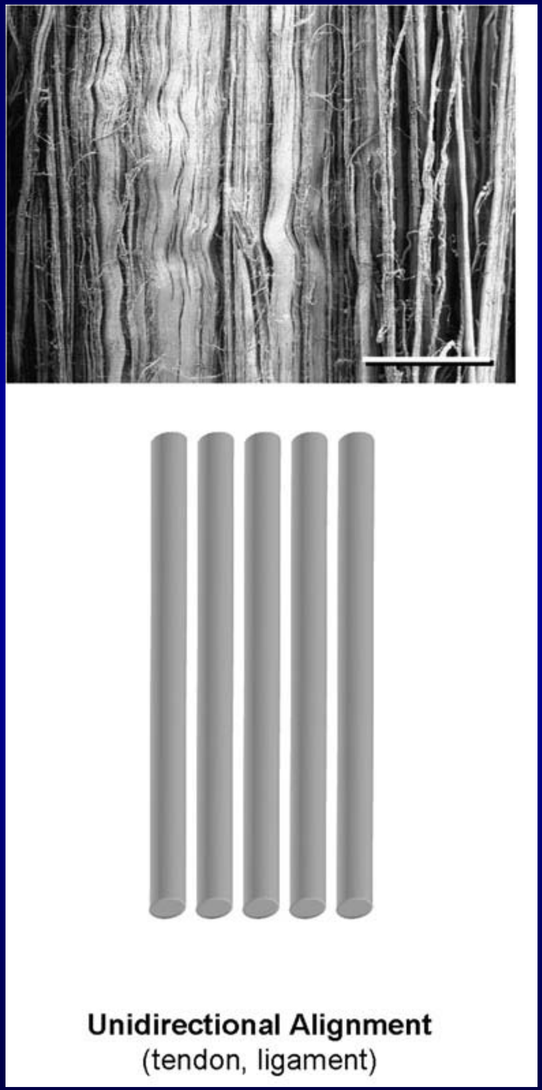

Image taken from Mauck *et al.*, 2009

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Background

- **Musculoskeletal soft tissue repair**
- **Tendons/ligaments**
  - **Aligned**
- **Annulus fibrosus**
  - **Opposing layers**

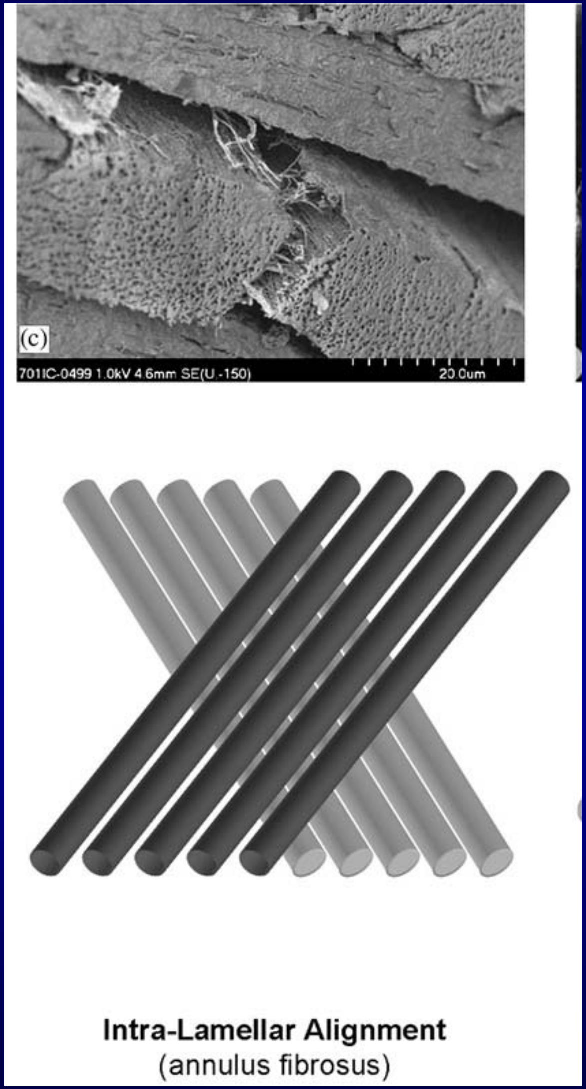


Image taken from Mauck *et al.*, 2009

Background

- **Musculoskeletal soft tissue repair**
- **Tendons/ligaments**
  - **Aligned**
- **Annulus fibrosus**
  - **Opposing layers**
- **Knee meniscus**
  - **Circumferential with perpendicular fibers**

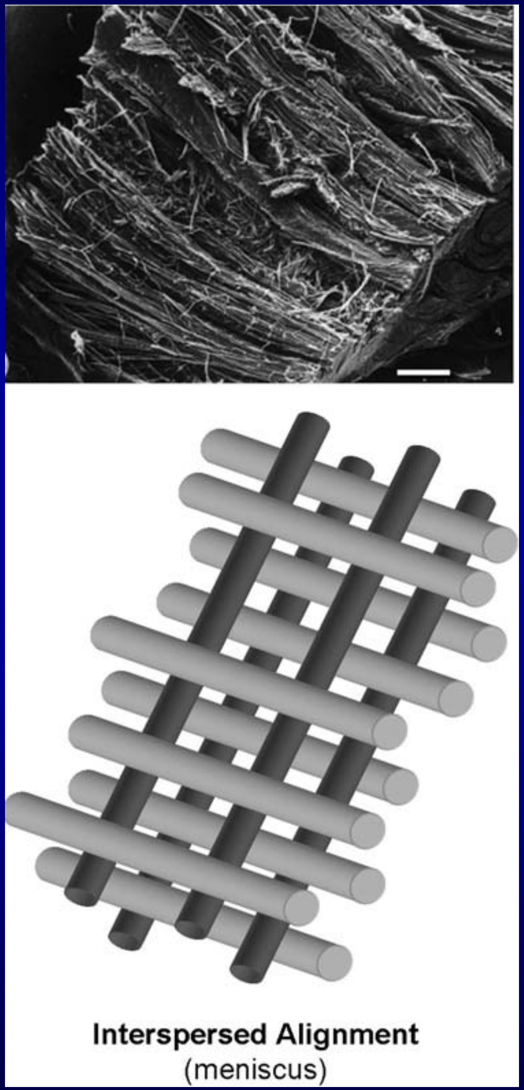
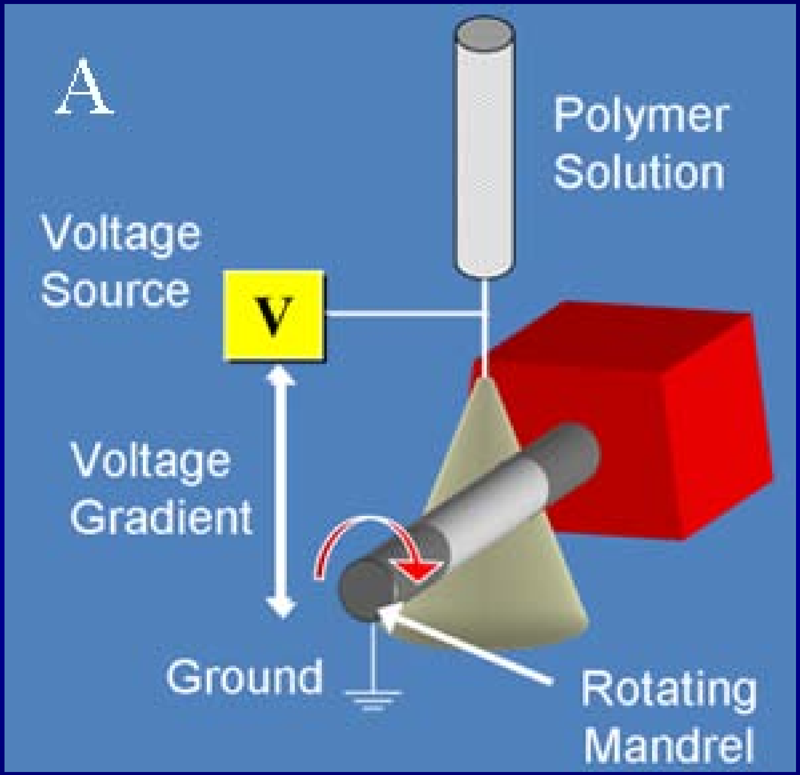


Image taken from Mauck *et al.*, 2009

Electrospinning



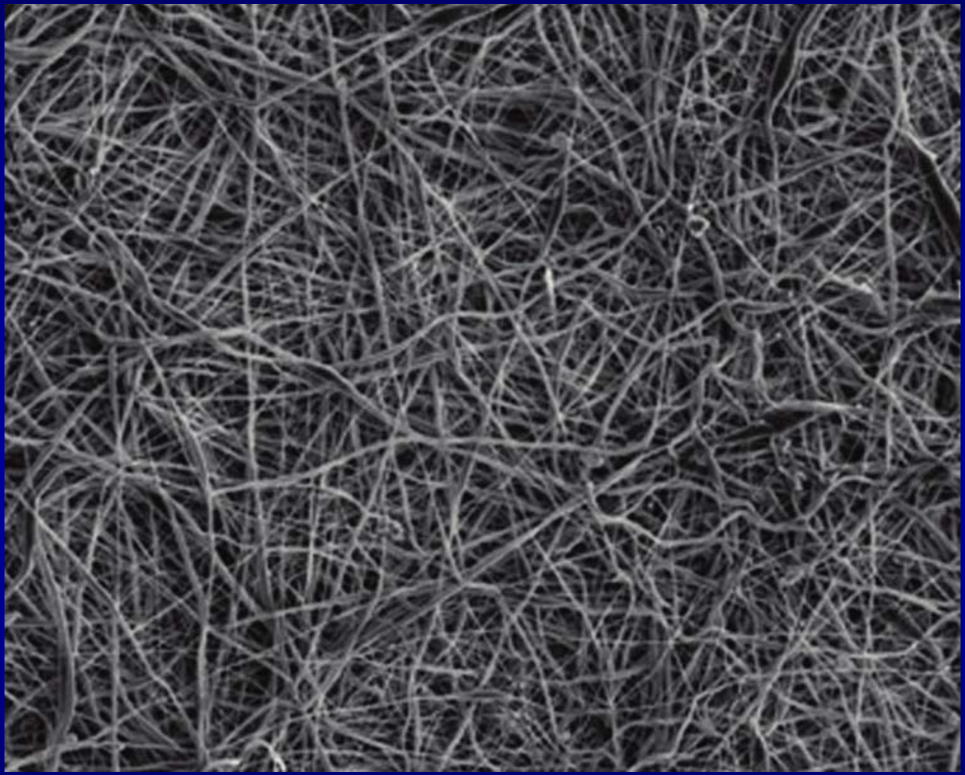
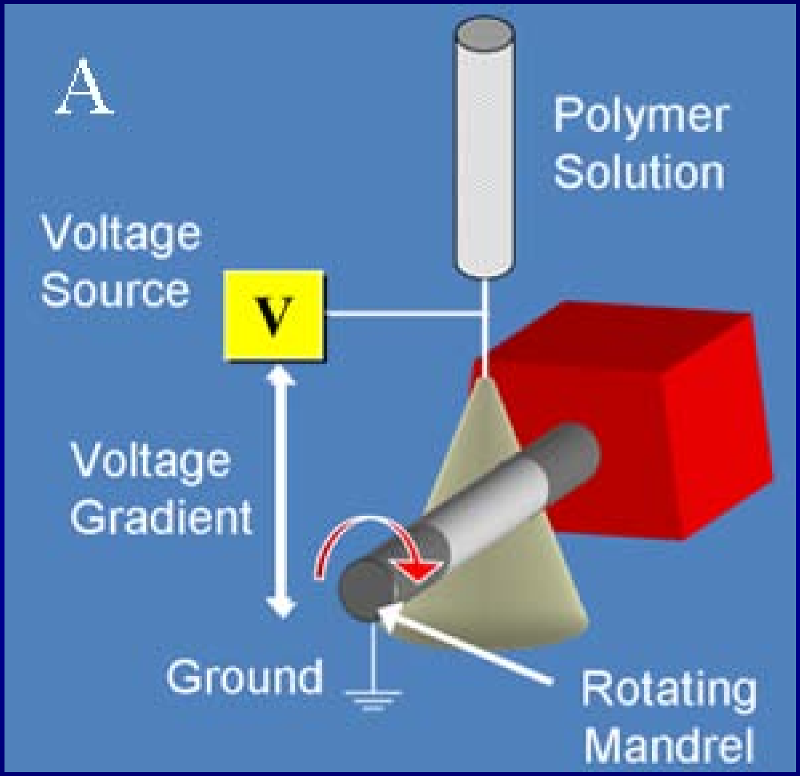
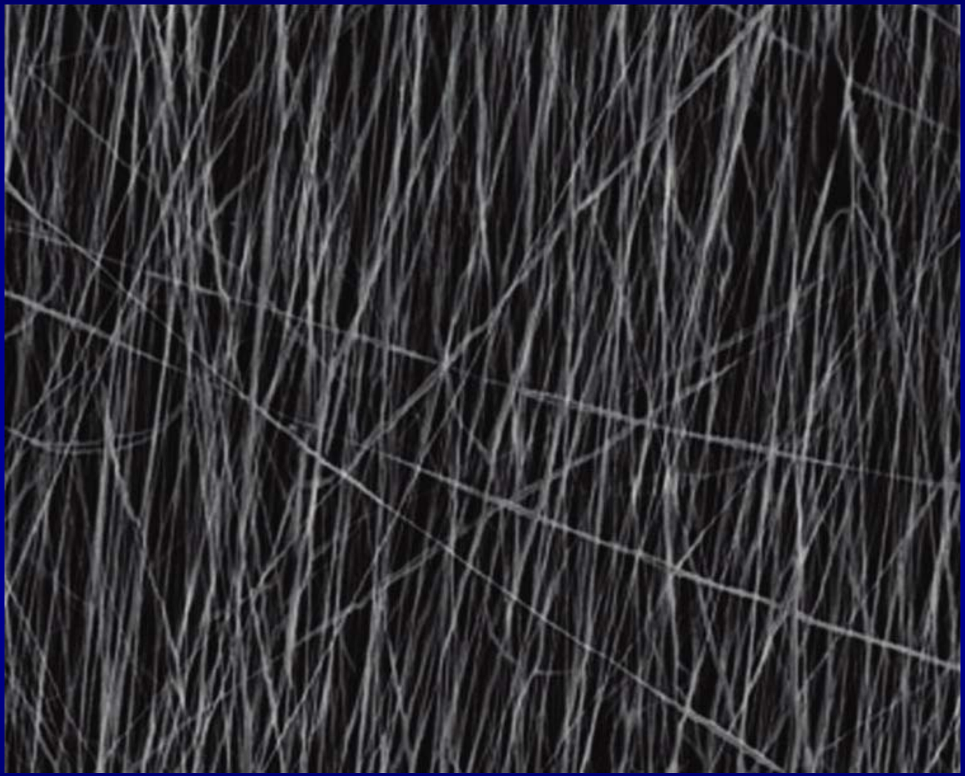


Image taken from Heo *et al*., Mauck *et al*., 2009

Electrospinning





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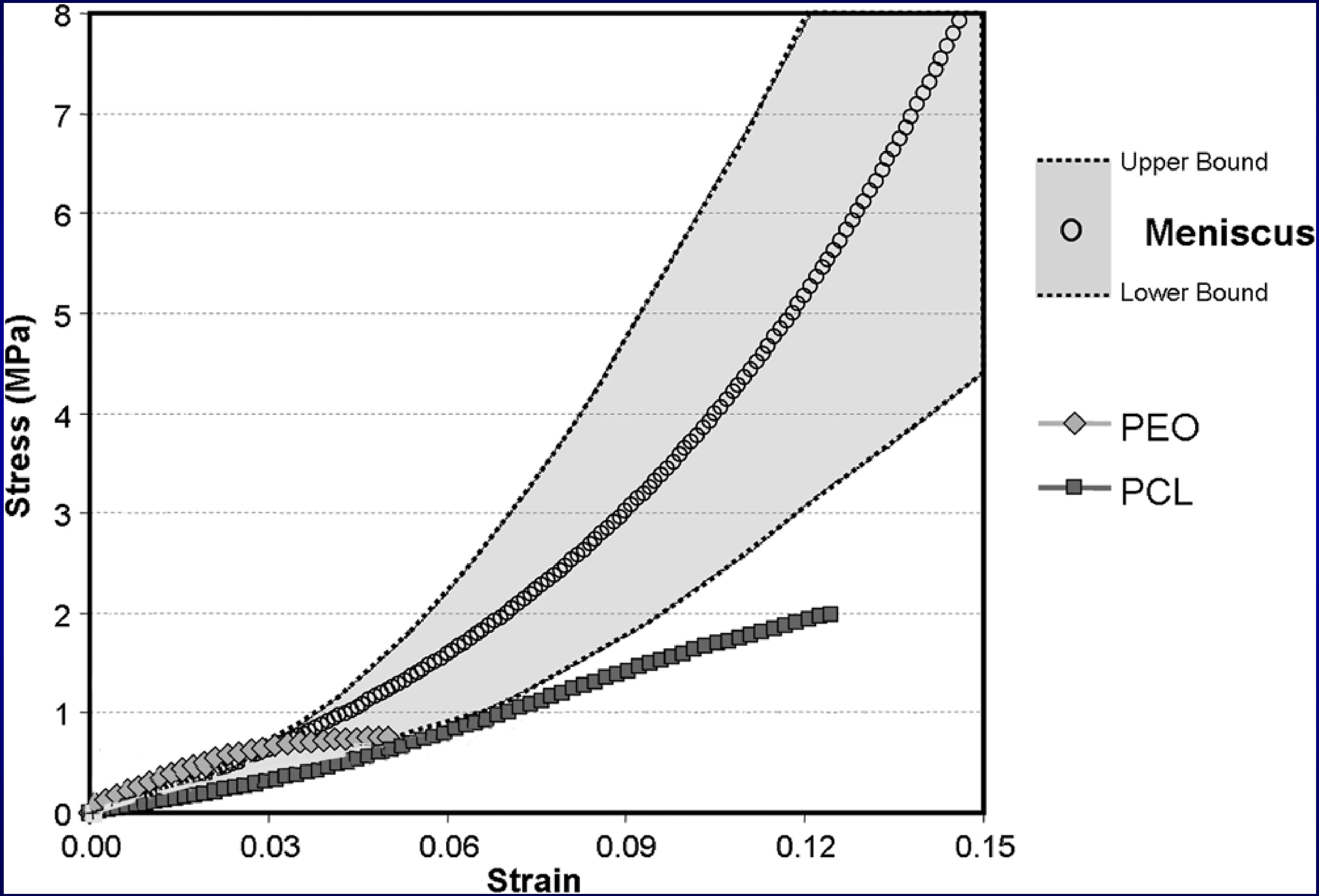
Synthetic scaffold mechanics



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Synthetic scaffold mechanics

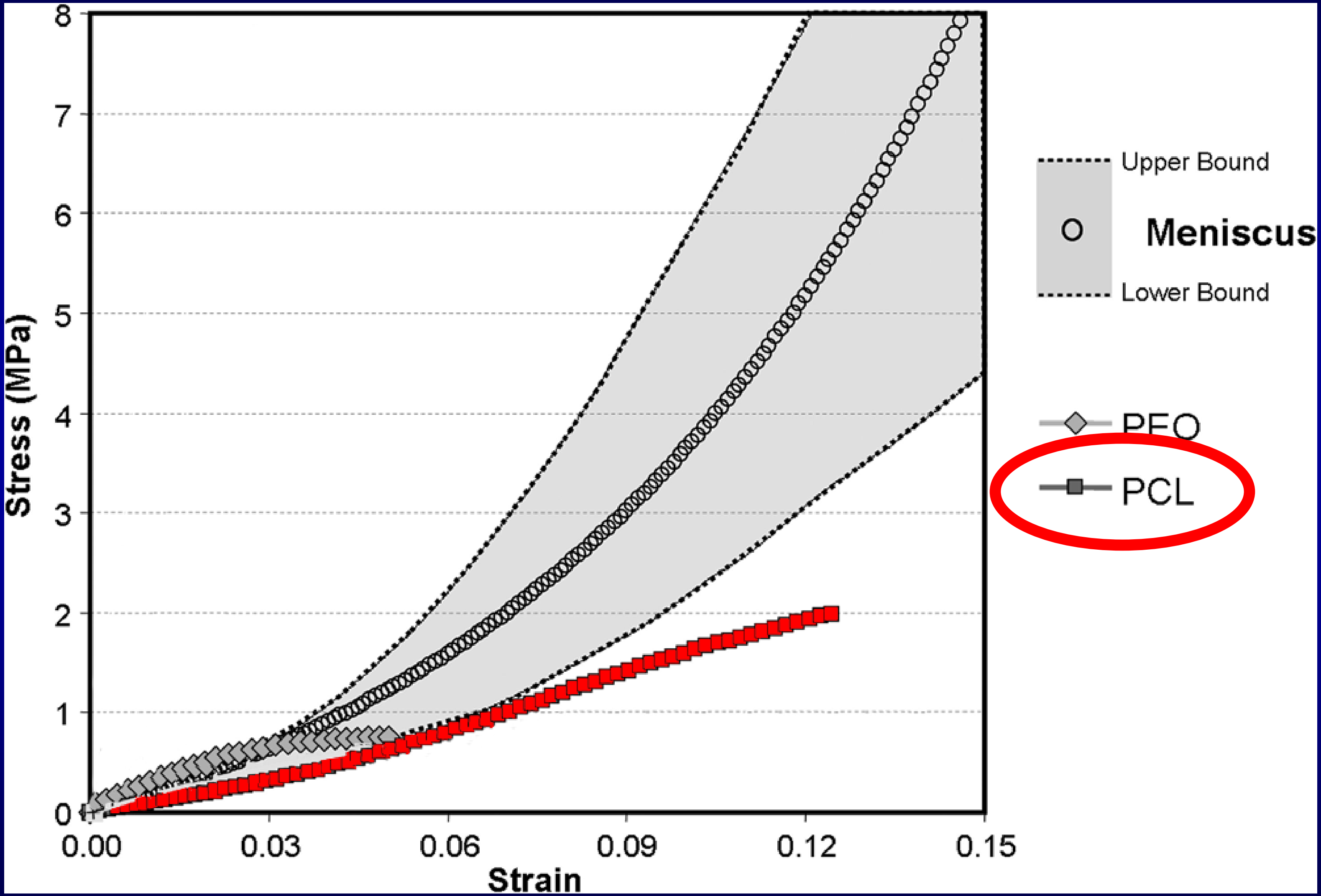

Image taken from Mauck *et al.*, 2009

Mesenchymal stem cells

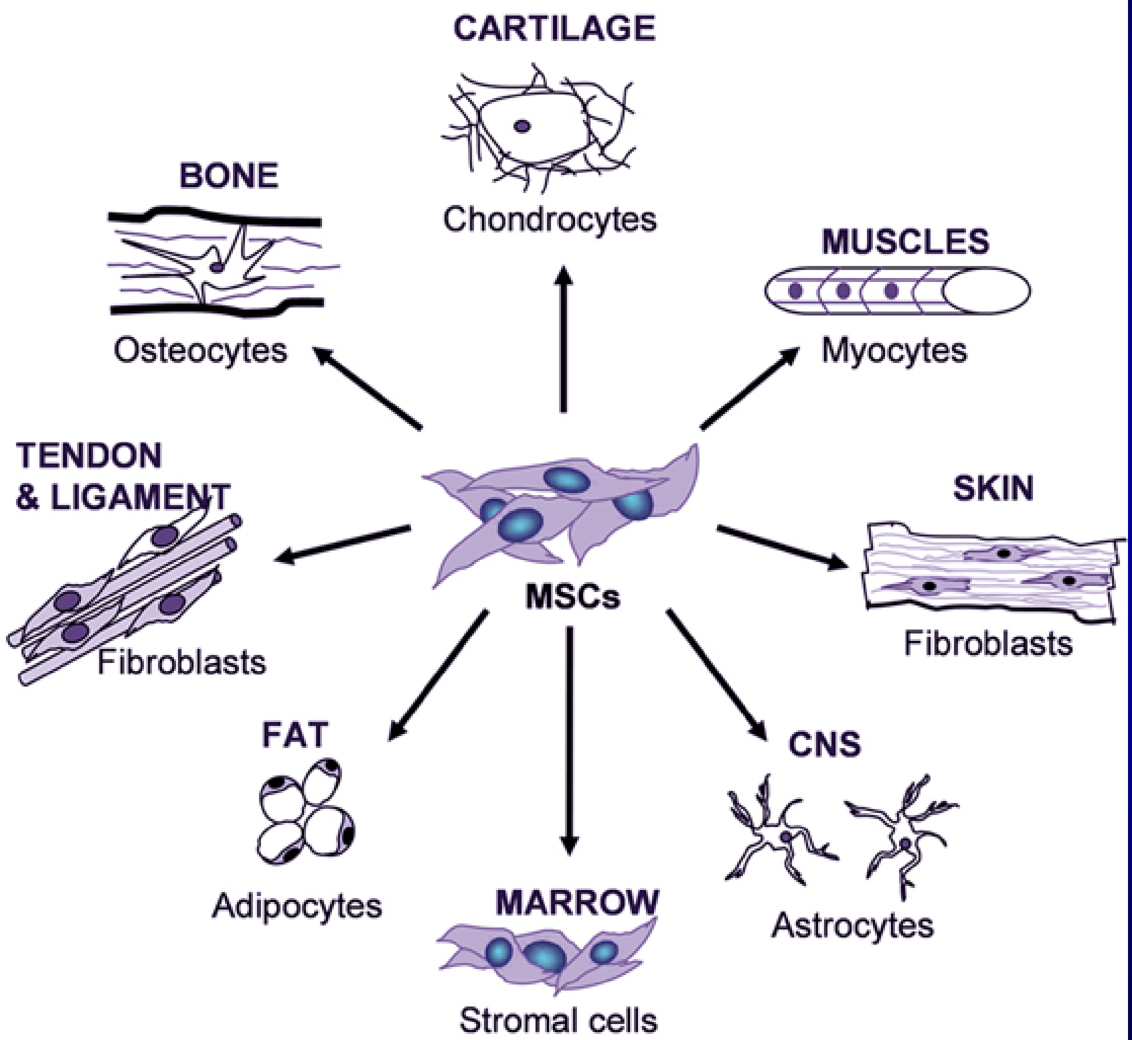

Image taken from http://www.xpand-biotech.com/technology_bioreactor.htm

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Mesenchymal stem cells

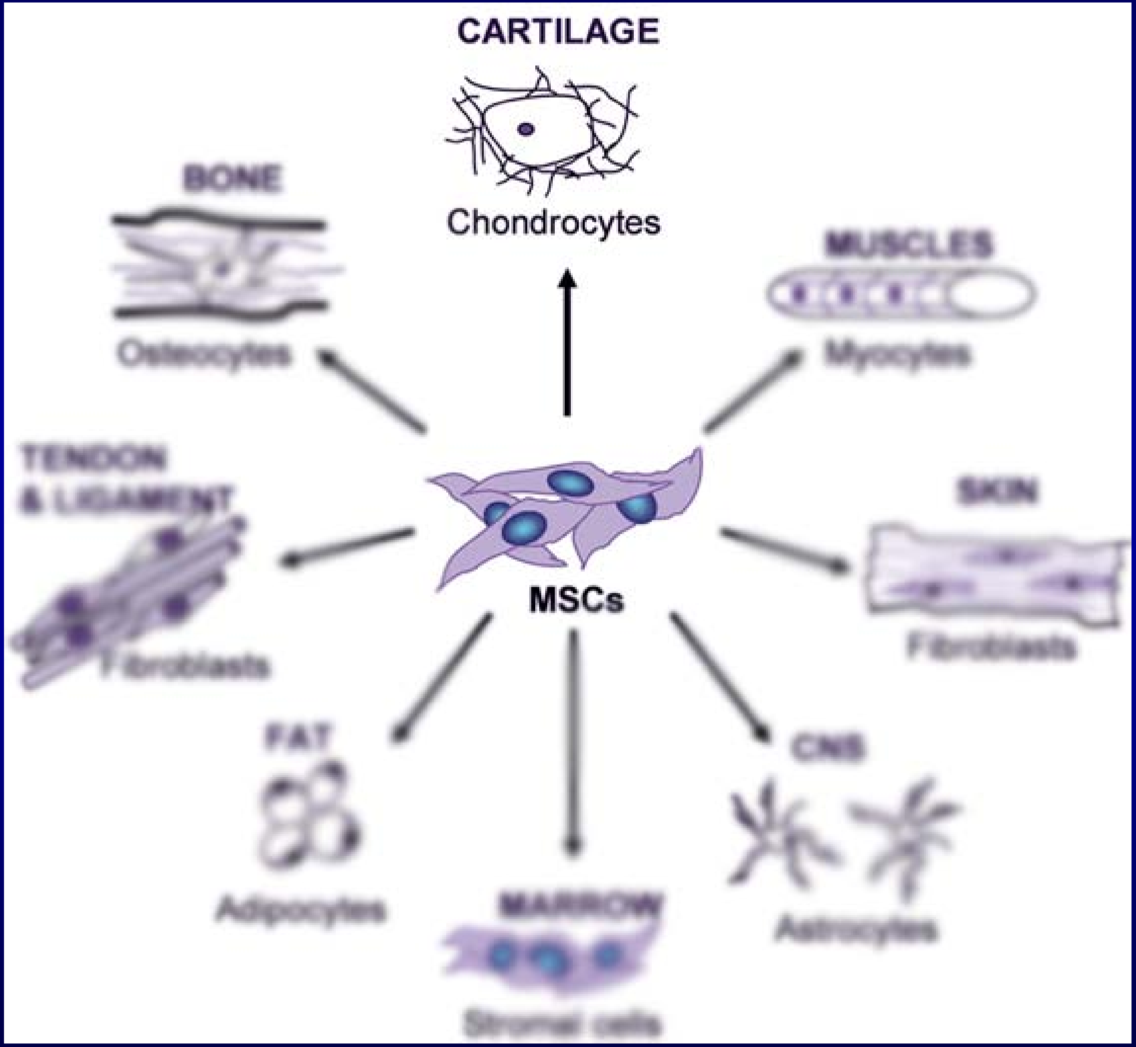

Image taken from http://www.xpand-biotech.com/technology_bioreactor.htm

MSC Differentiation

- **Chemical cues**
  - **TGF-β1 / β3 and BMP-2** **(Noth *et al.*, 2007; Barry *et al.*, 2001; Schmitt *et al.*, 2003)**

- **Physical cues**
  - **Dynamic loading** **(Huang *et al.*, 2004)**
  - **Substrate stiffness** **(Engler *et al.*, 2006)**

MSC Differentiation

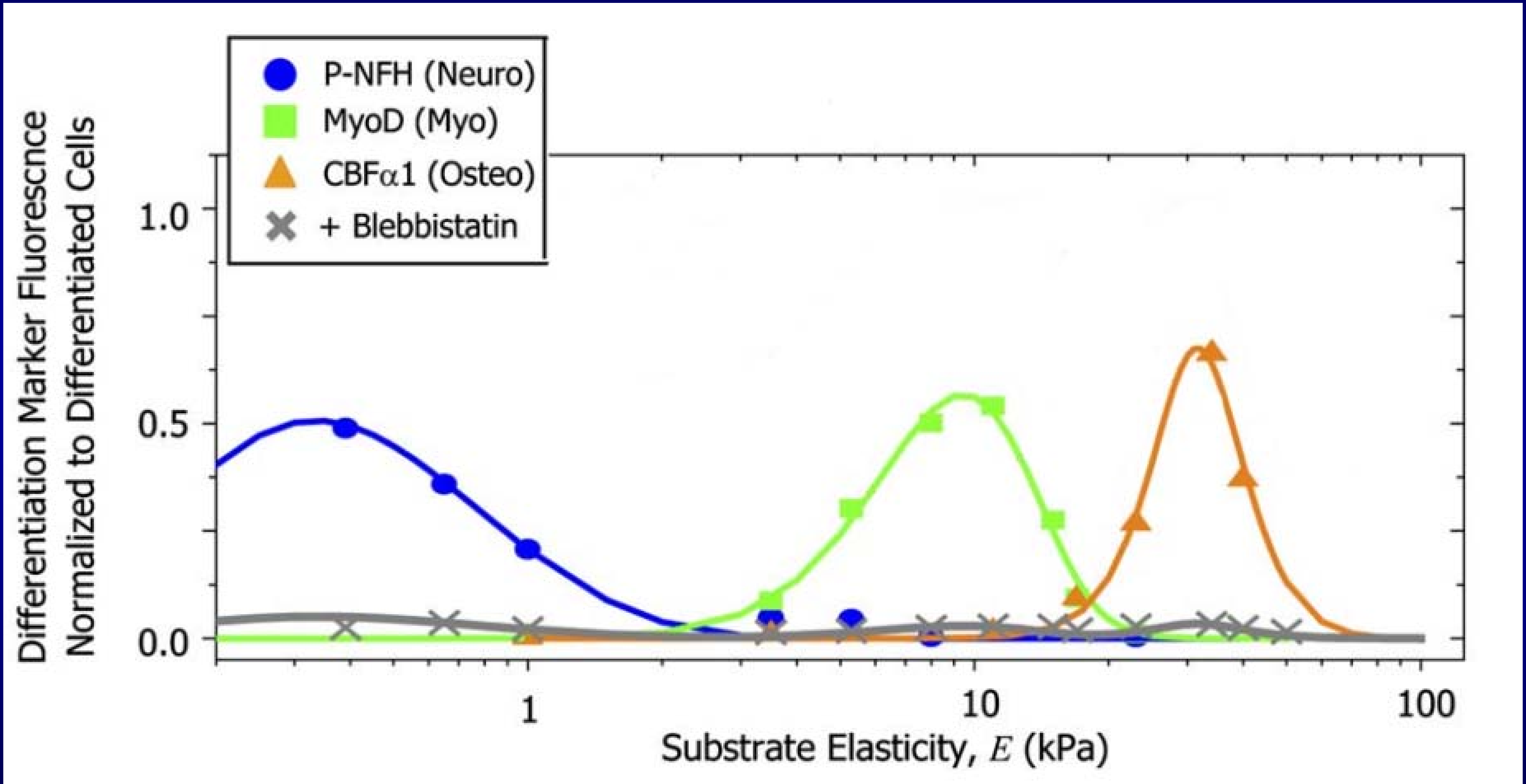

Image taken from Engler *et al*., 2006

Cells as sensors

- **Extracellular strain is transmitted from exterior of the cell to the nucleus via various cellular structures (FAK, actin cytoskeleton, lamin, nesprin)** (Nathan *et al.*, 2011; Rezzonico *et al*., 2003; Lammerding *et al*., 2004; Chancellor *et al.*, 2010)
- **Changes in gene expression accompany nuclear deformation** (Heo *et al.*, 2011; Hoshiba *et al*., 2008)

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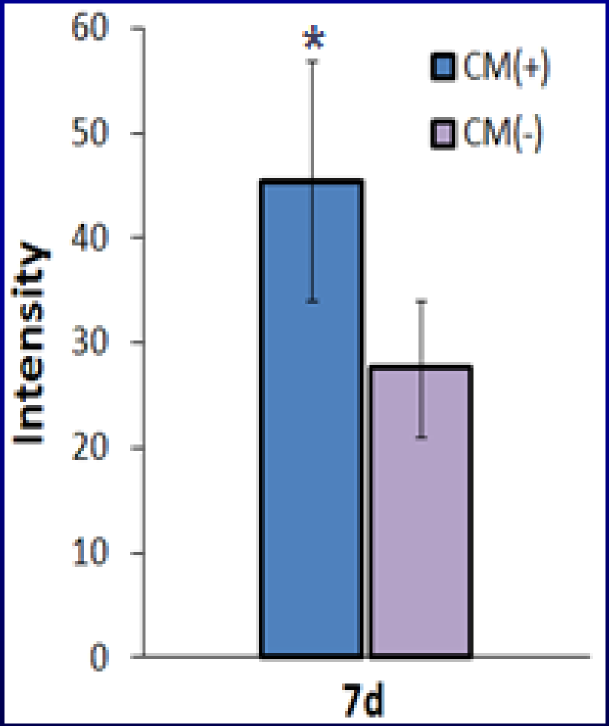
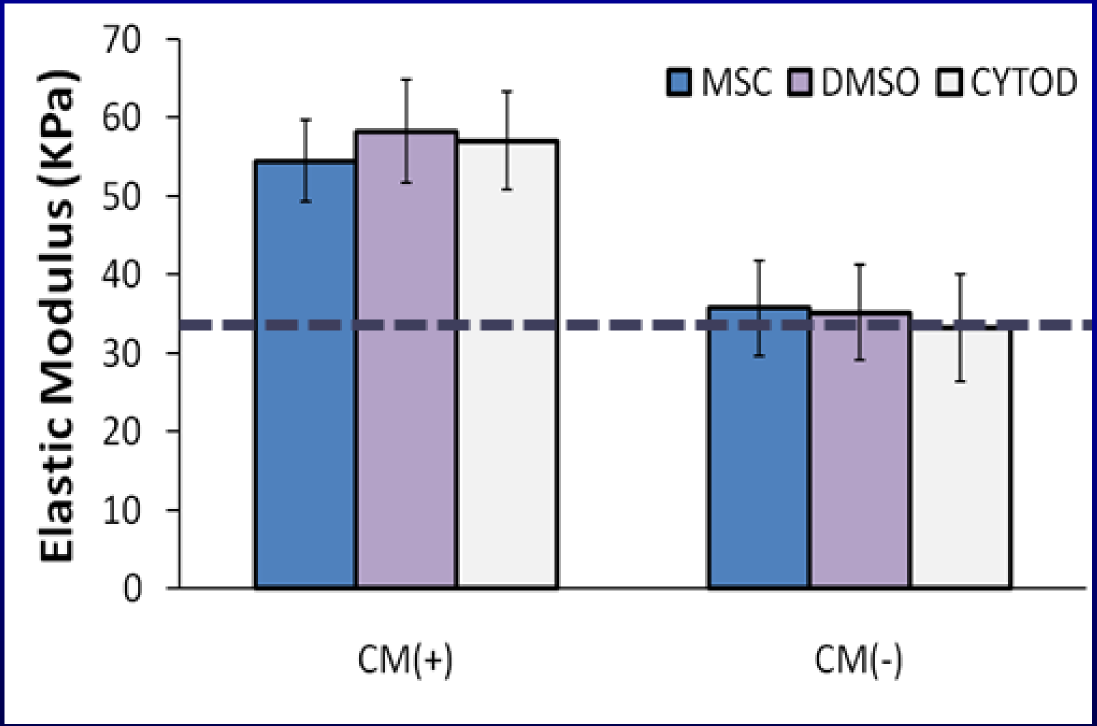
Nuclear mechanics

- **Increasing heterochromatin concentration levels accompanies nuclear stiffening**





Heo *et al.*, 2011

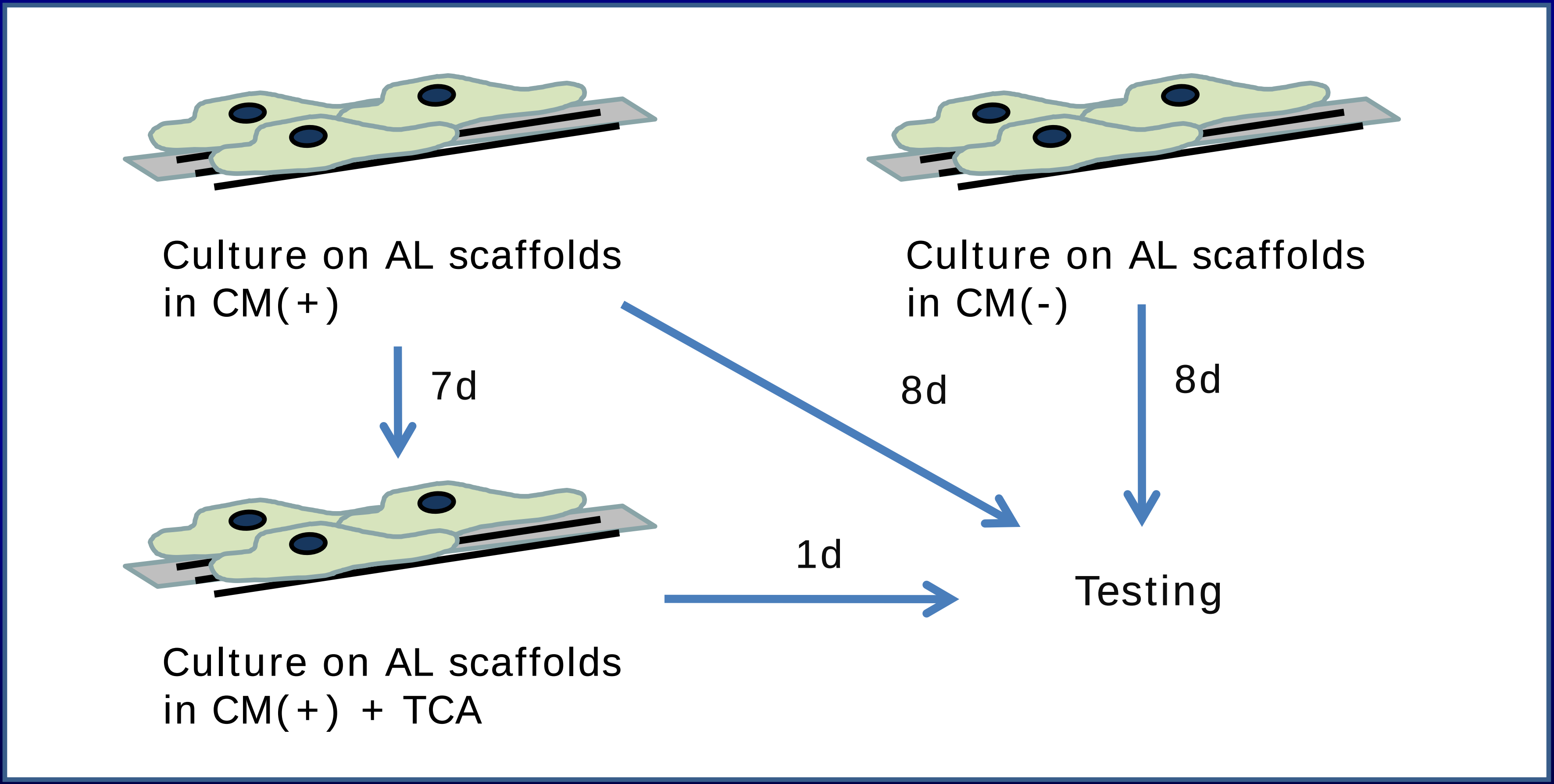
Our starting point…

- **What happens to MSC's response to mechanical stimuli when nuclear mechanics are altered?**

- **Trichostatin A**
  - **Histone deacetylase inhibitor (Yoshida *et al.*, 1995)**
  - **Results in chromatin relaxation and less heterochromatin (Toth *et al.*, 2004)**

Experimental design

- **Mesenchymal stem cells**
  - Juvenile bovine MSCs from tibial and femoral bone marrow



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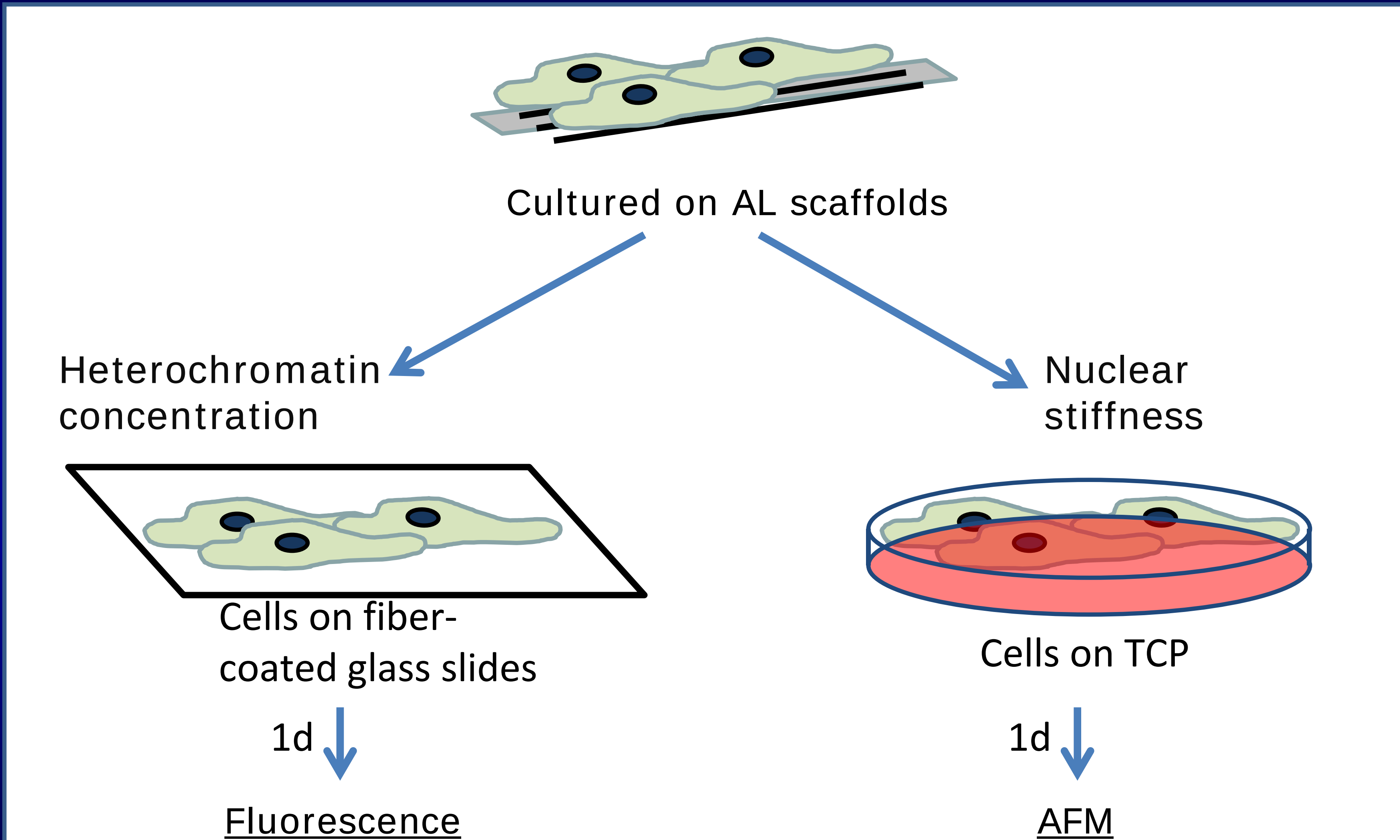
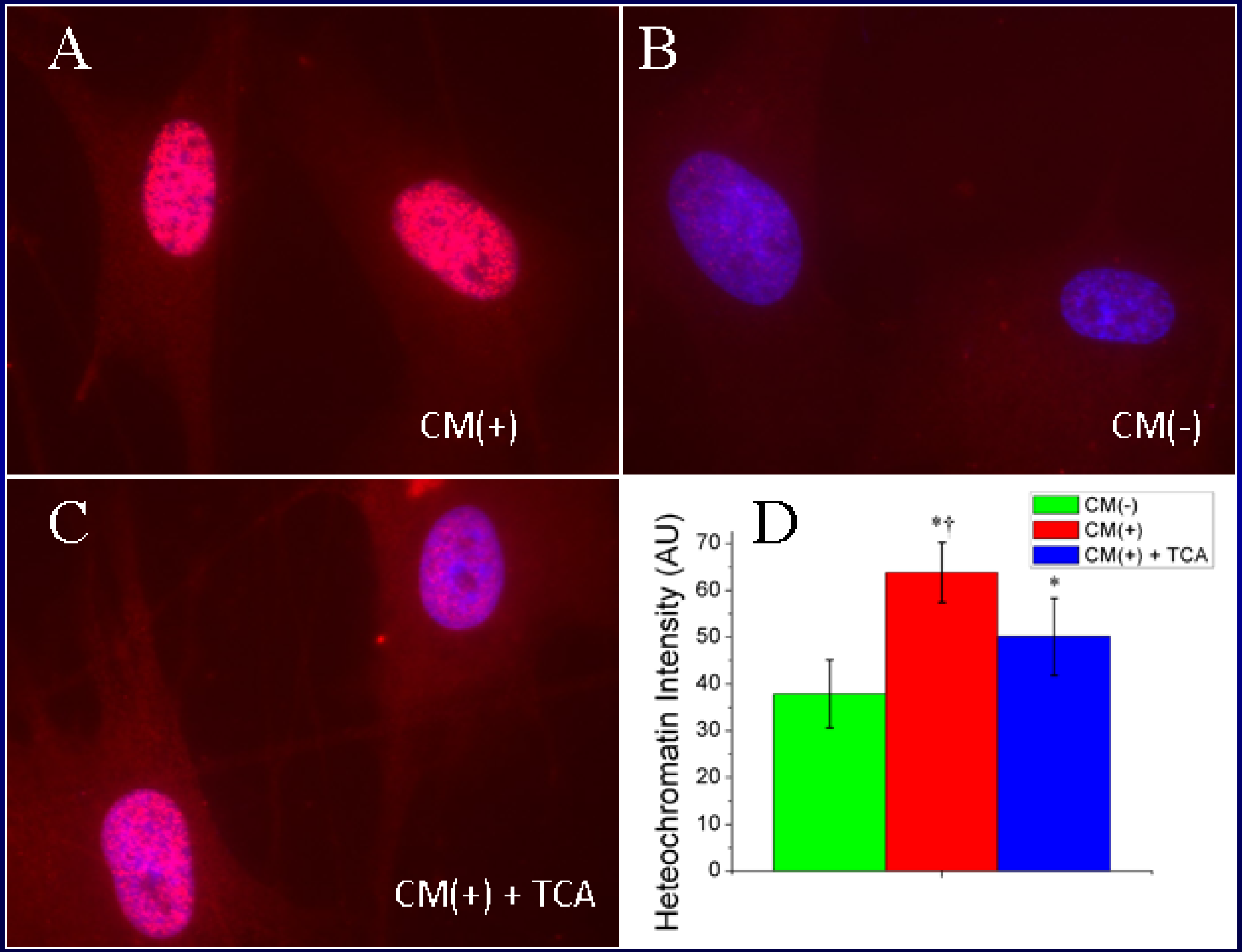
Experimental design



Heterochromatin condensation



* P < 0.01 vs. CM(-). † P < 0.01 vs. CM(+) + TCA. n = 35

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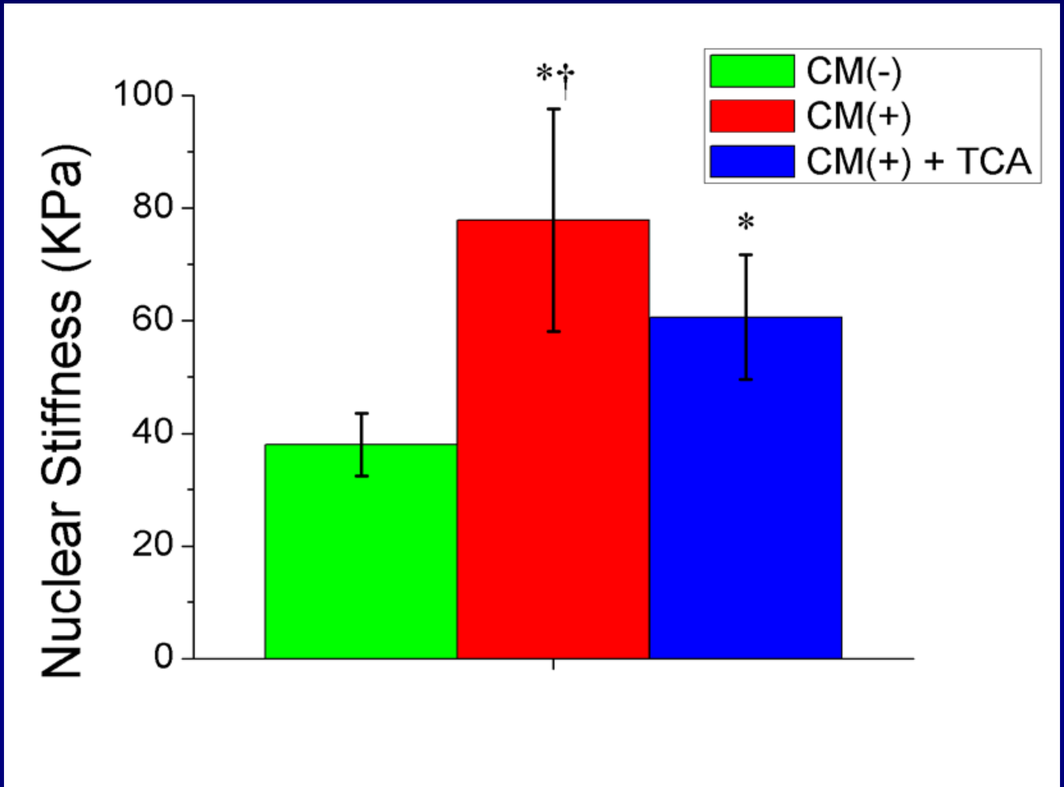
Nuclear stiffness



* P < 0.01 vs. CM(-).
† P < 0.01 vs. CM(+) + TCA .
n = 10

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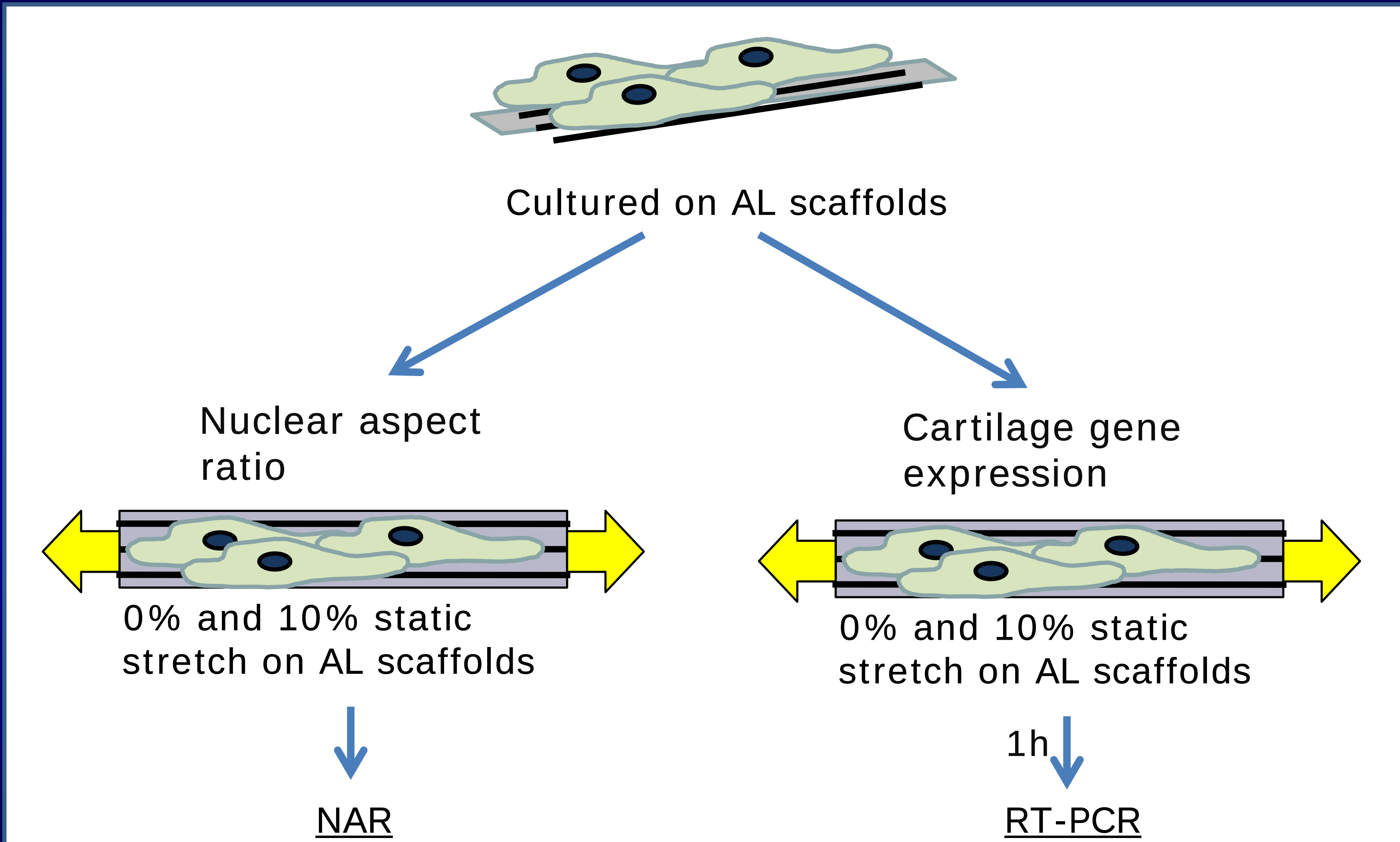
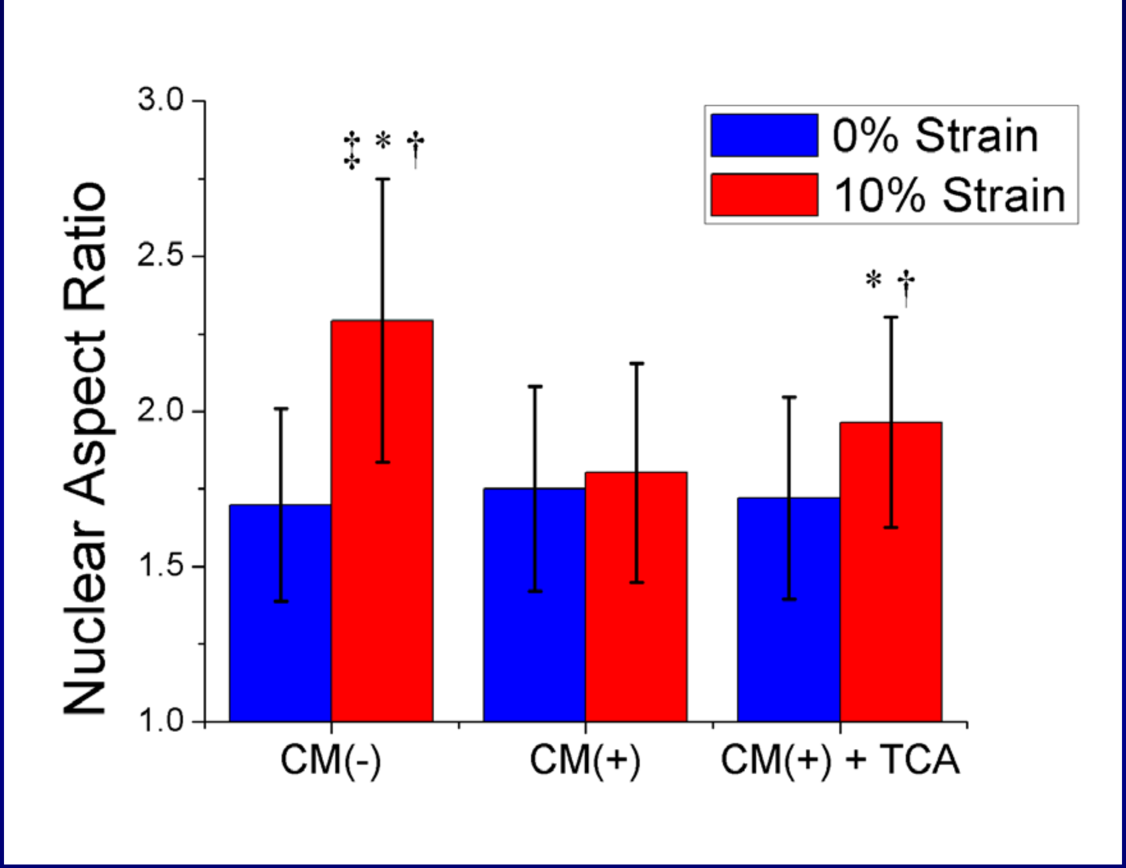
Experimental design



Nuclear aspect ratio



* P < 0.01 vs. same culture with 0% strain.
‡ P < 0.01 vs. CM(+) + TCA with 10% strain.
† P < 0.01 vs. CM(+).
n ≥ 200

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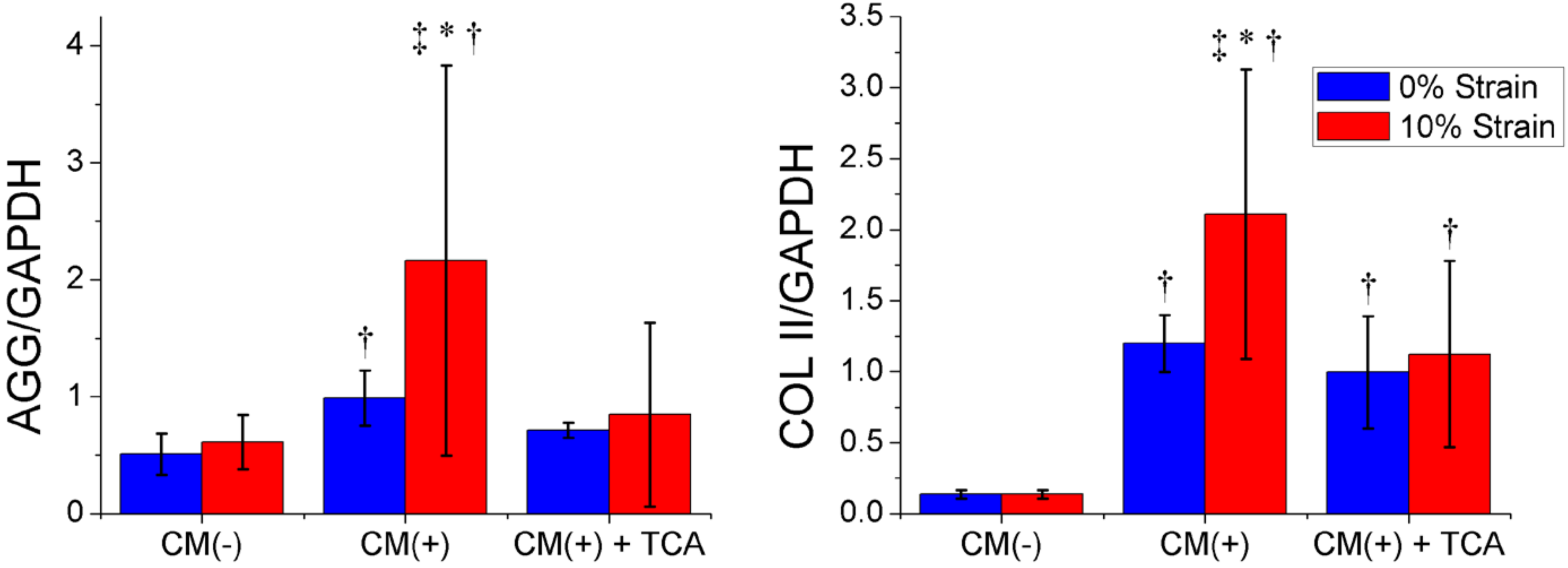
Cartilage gene expression



* P < 0.05 vs. 0% stretch.
† P < 0.05 vs. CM(-).
‡ P < 0.05 vs. CM(+) + TCA.
n = 5

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Conclusion

- **A decrease in nuclear stiffness can be seen to accompany decreasing heterochromatin concentrations** (consistent with increasing stiffness with differentiation Pajerowski *et al.*, 2007)
- **Altered nuclear mechanics due to heterochromatin condensation affects MSCs response to mechanical stimuli**
- **This can be seen in the increased NAR during static stretch conditions**
- **AGG and COL II are no longer up-regulated with stretch** (consistent with TCA inhibits differentiation Lee *et al.*, 2004)

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Acknowledgements

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  - Dr. Rob Mauck
  - Su-Jin Heo
  - Tristan Driscoll
  - Everyone
- Dr. Jan Van der Spiegel

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WHERE DISCOVERIES BEGIN

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DISORDERS

NIBIB

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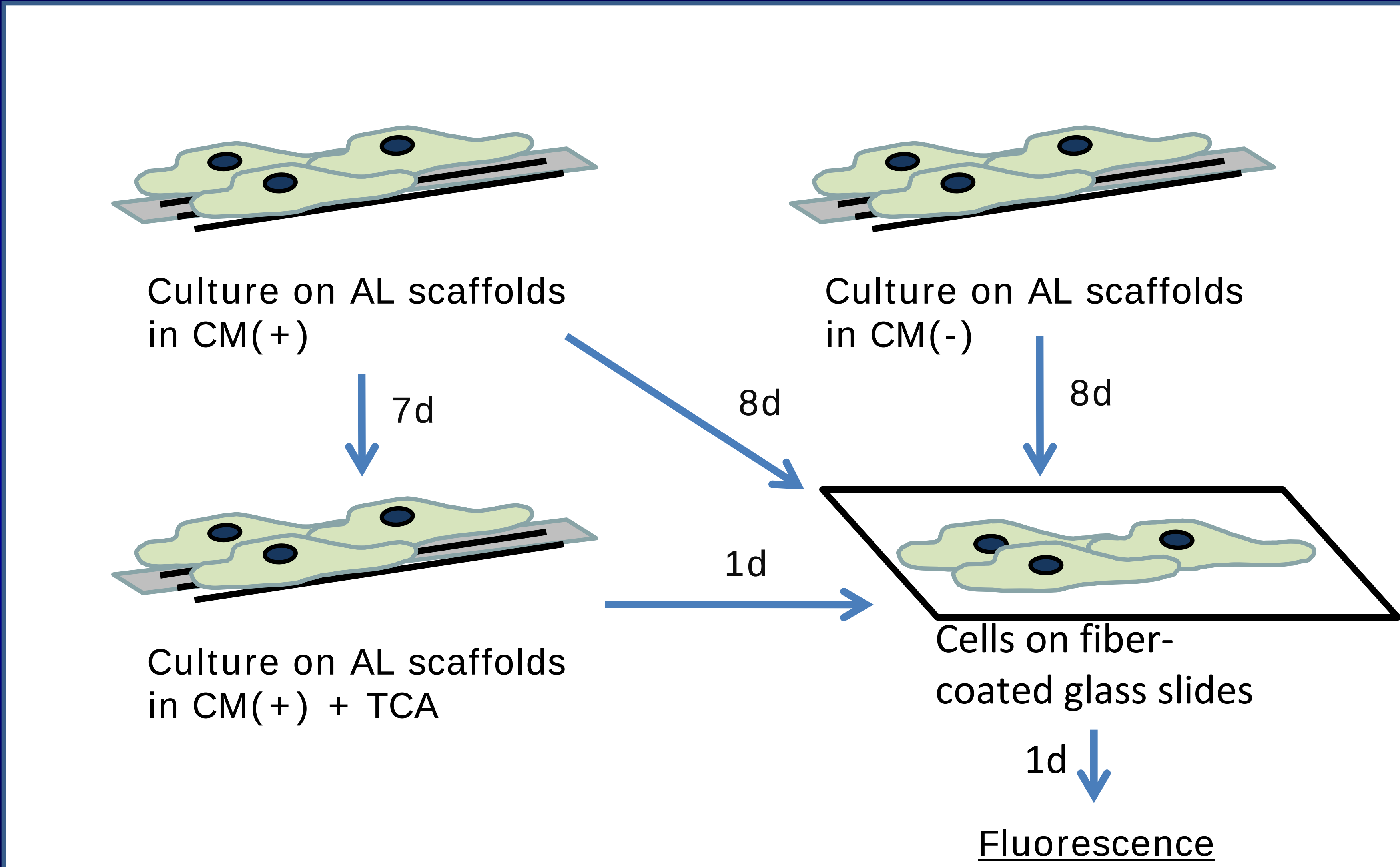
Heterochromatin concentration



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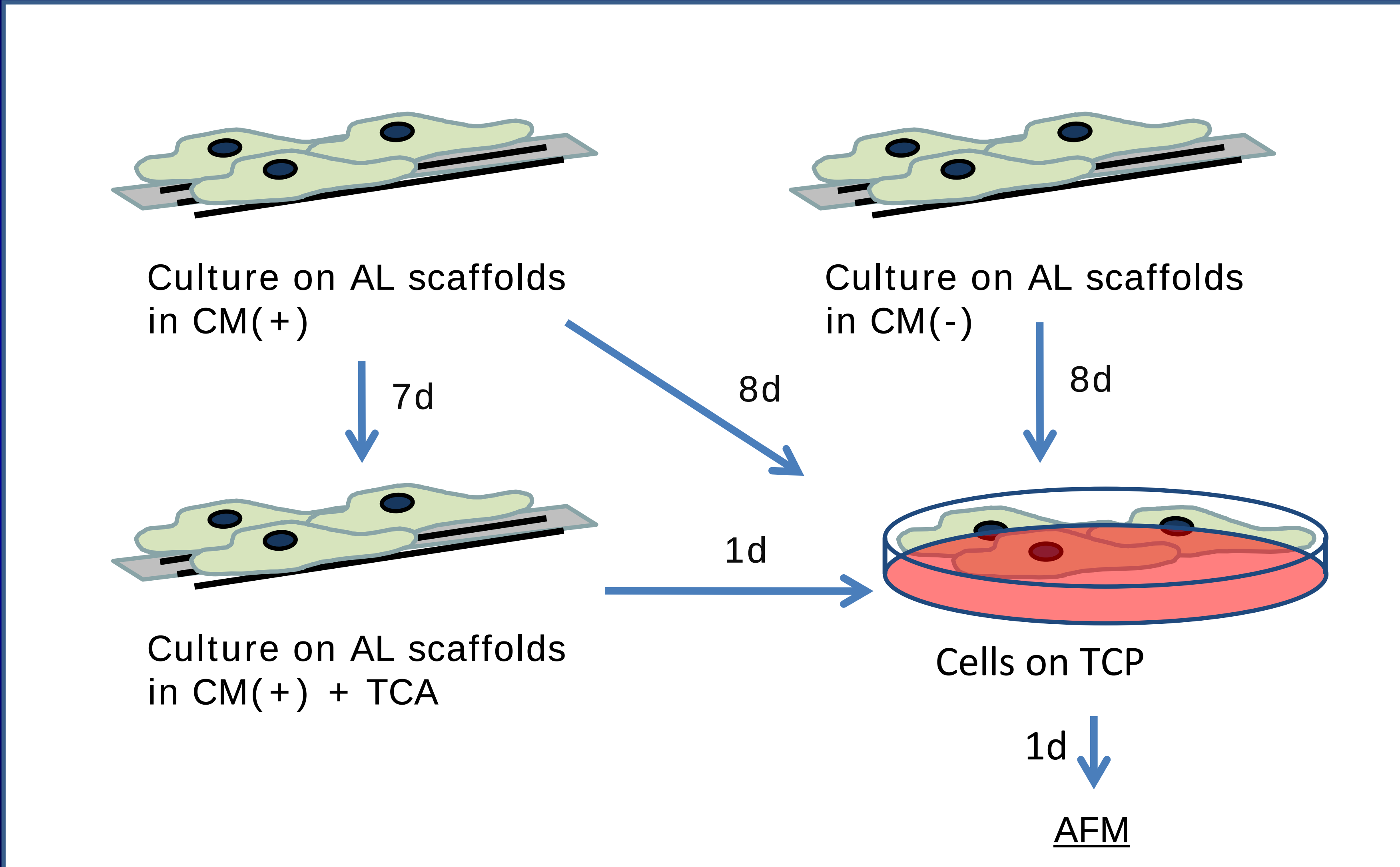
Nuclear stiffness



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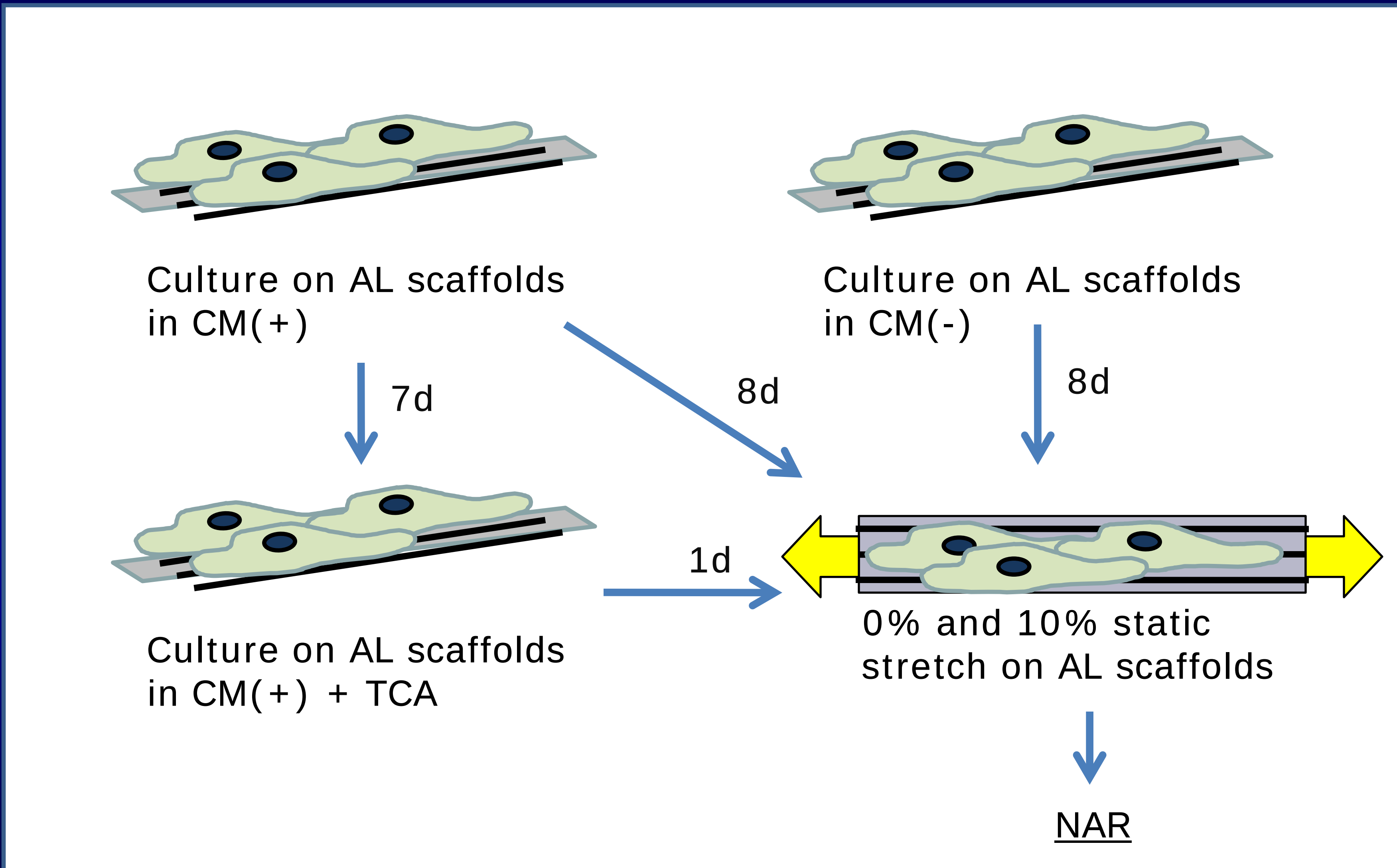
Nuclear aspect ratio



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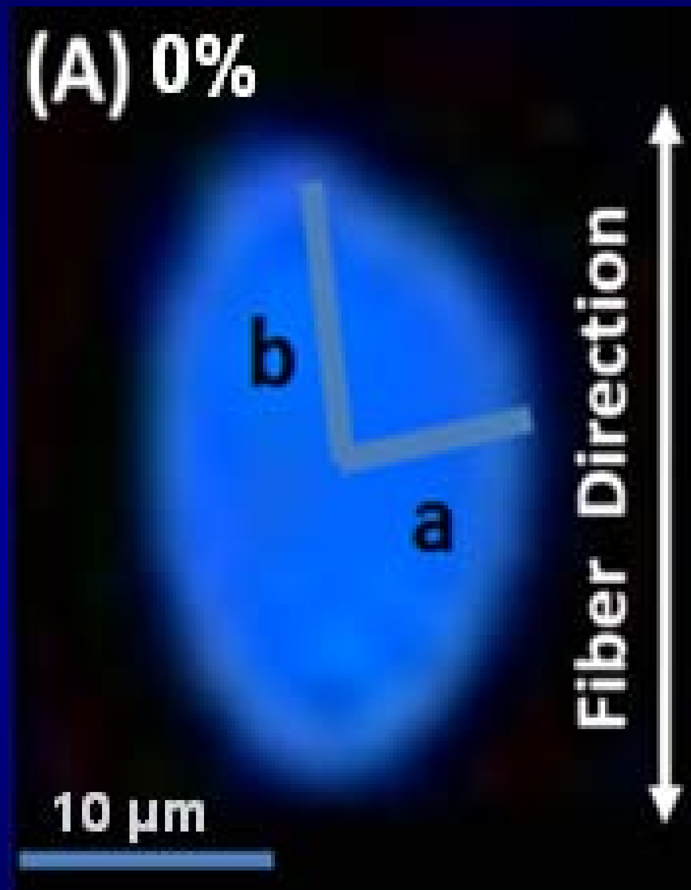
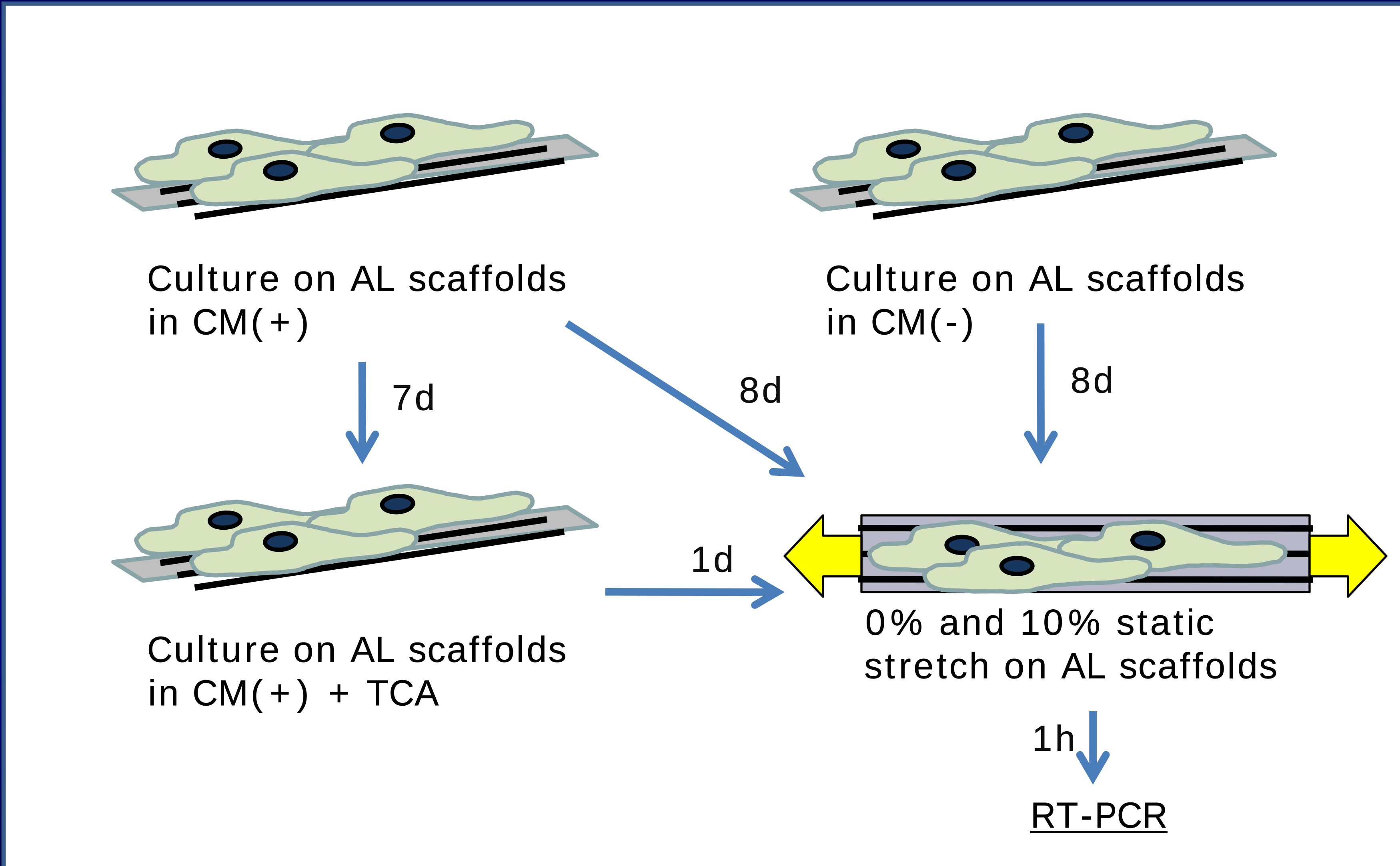


Heo *et al.*, 2011

Cartilage gene expression



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