

Mechanical Stimulation of Enteric Smooth Muscle

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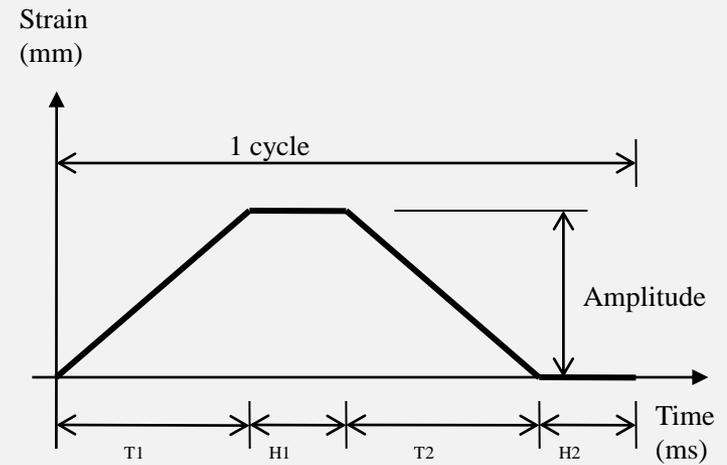
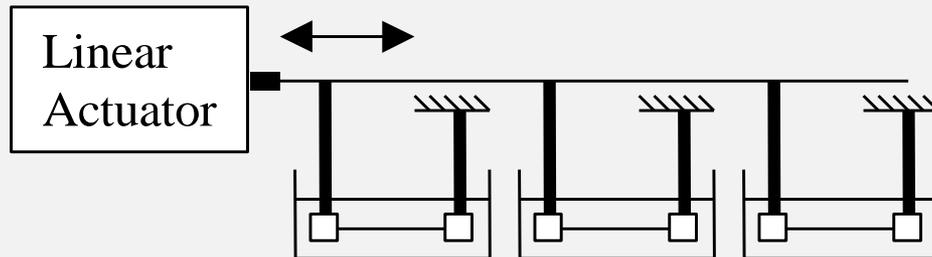
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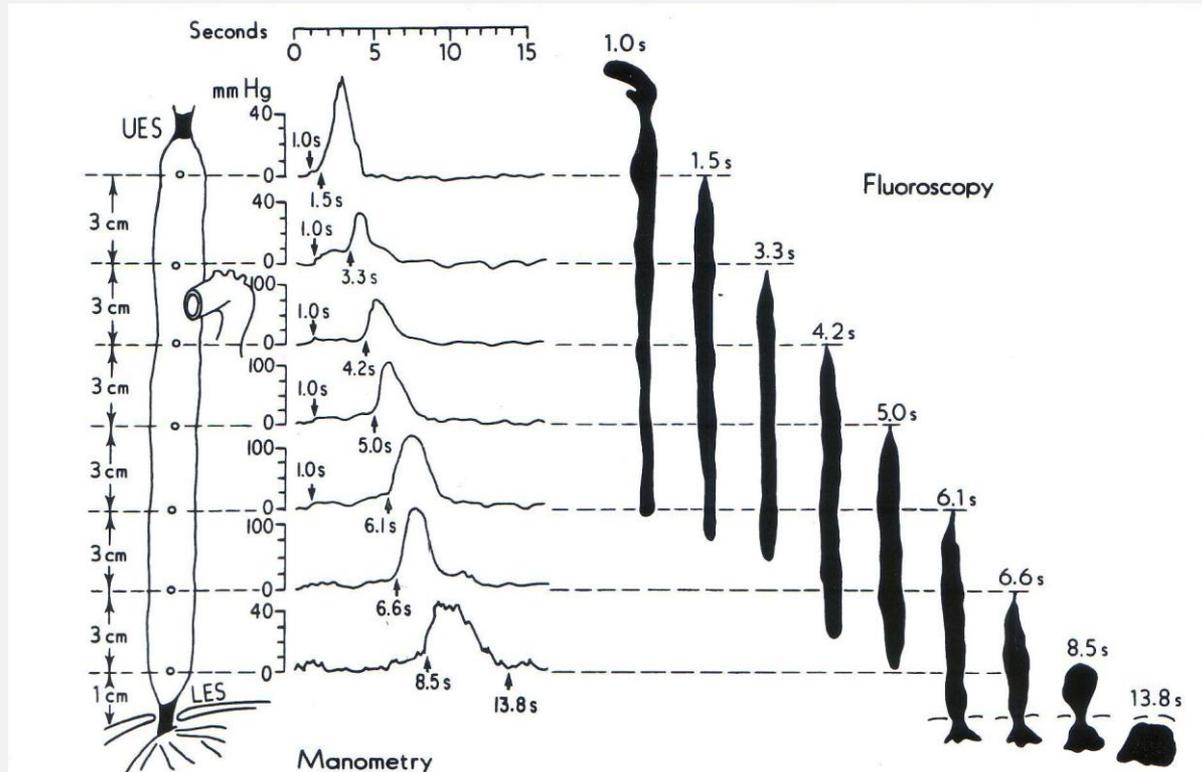
Objectives

- To examine the effects of mechanical forces on oesophageal smooth muscle cells *in vitro*
 - Alignment
 - Phenotype
- To optimise the chemico-mechanical environment for *in vitro* development of cell-scaffold constructs

Bioreactor



Oesophageal swallowing

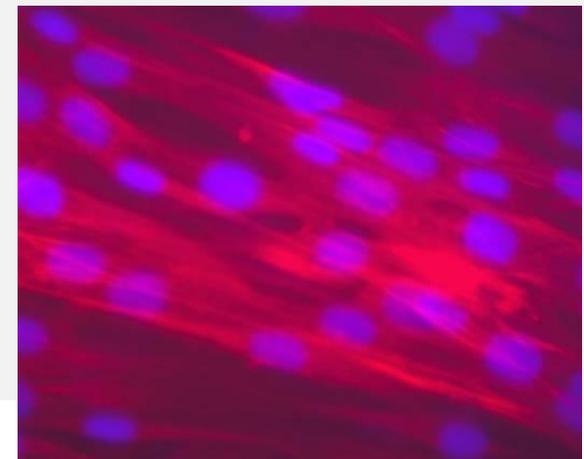
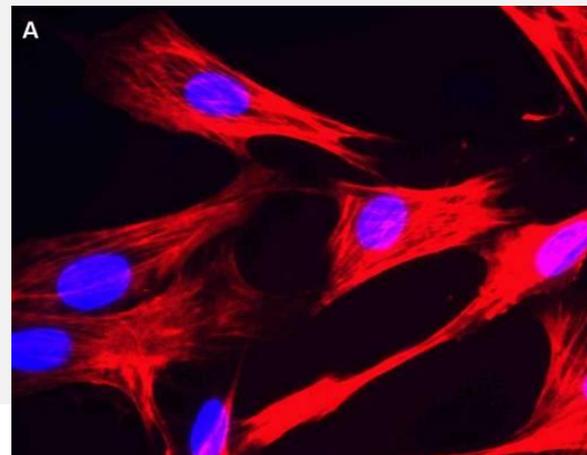
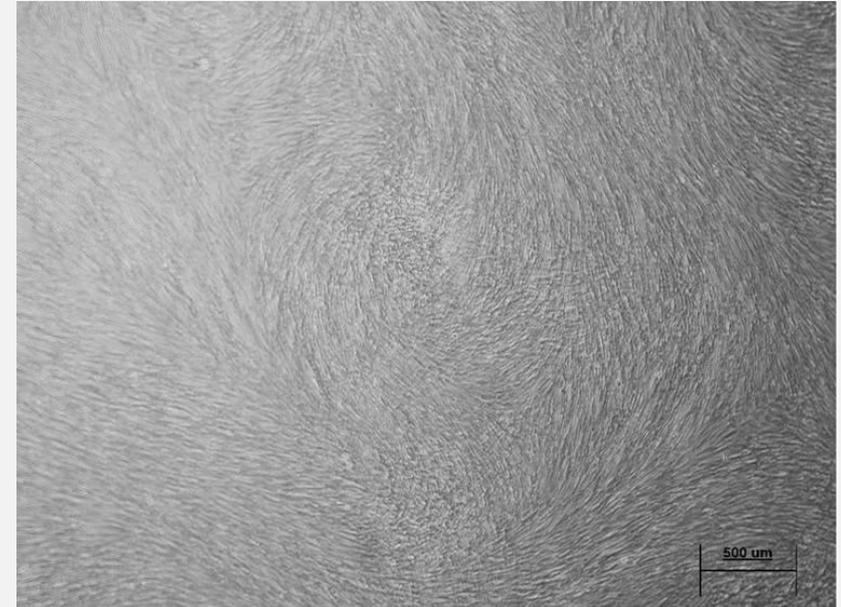


Cell Culture

- Smooth muscle cells isolated from the muscularis mucosae of the abdominal oesophagus of mature swine
- Used for experiments between passages 4 and 10
- Seeded onto scaffold and maintained in static culture for 2 days before onset of stimulation

Primary Culture of Porcine Smooth Muscle Cells

- Cell Phenotype
 - Hill-valley growth pattern
 - Actin Positive Expression



- Materials:
 - Air dried Chitosan
 - Chitosan-Collagen Blends
 - Chitosan-Collagen with Genipin crosslinking
 - PU & tegaderm with and without O₂ plasma treatment
- O₂ Plasma treated PU selected:
 - Repeatability
 - Elasticity
 - Cytocompatibility



Cells growing on O₂ treated PU sheet
(200W 60s) at day 14

1D Stimulation of Smooth Muscle Cells

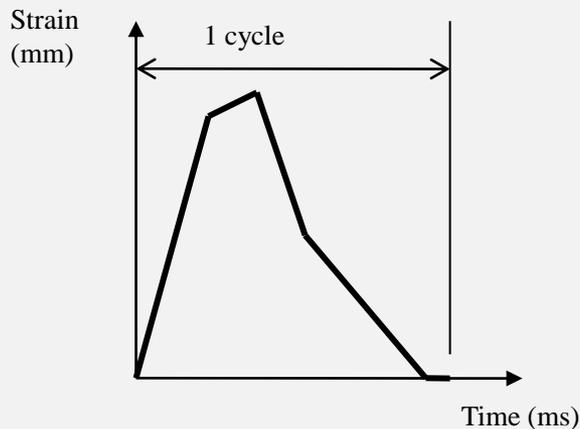
- Method

- Cells seeded at density of 5×10^4 per 1 sample plasma treated PU (2 days of cell attachment)

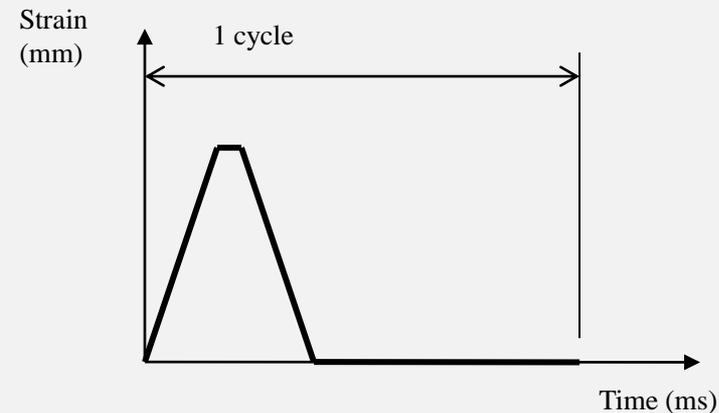
- Stimulation:

- a. Cardiovascular cycle: 1Hz (sinusoidal) for 6 hrs per day

- b. Oesophageal cycle¹: 1 cycle every 13.8 s, 400 cycles per day



(a)

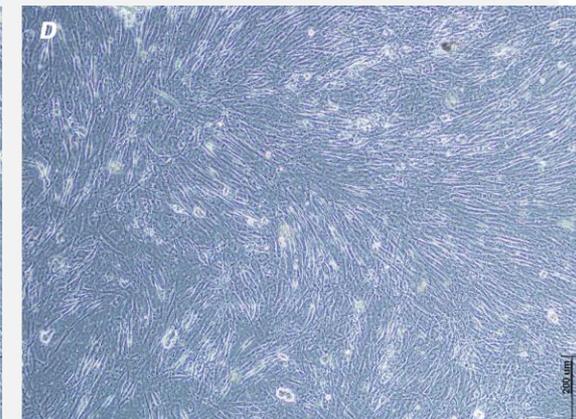
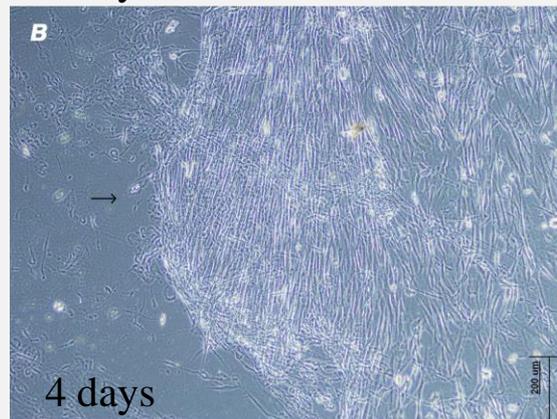
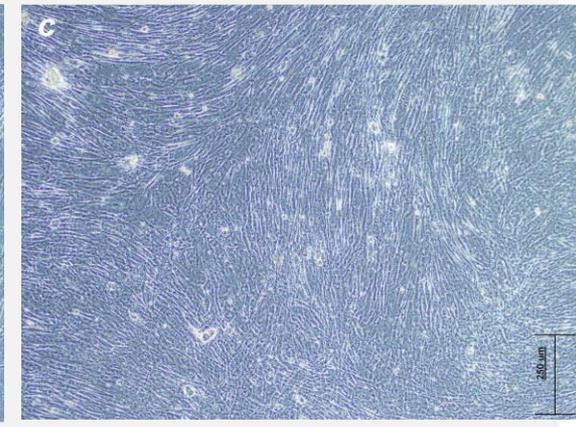
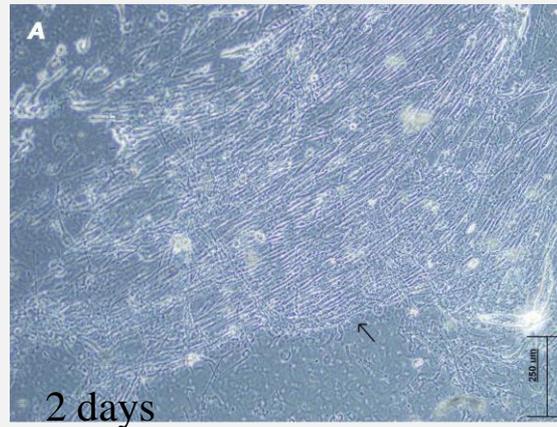


(b)

¹Castell, D.O. (1995). *The Esophagus* (2nd ed.). USA: Little, Brown and Company

1D Stimulation of Smooth Muscle Cells

- Cardiovascular cycle at 10% strain induces cell alignment perpendicular to the direction of strain after 2 days of stretching. Large areas of cell loss observed

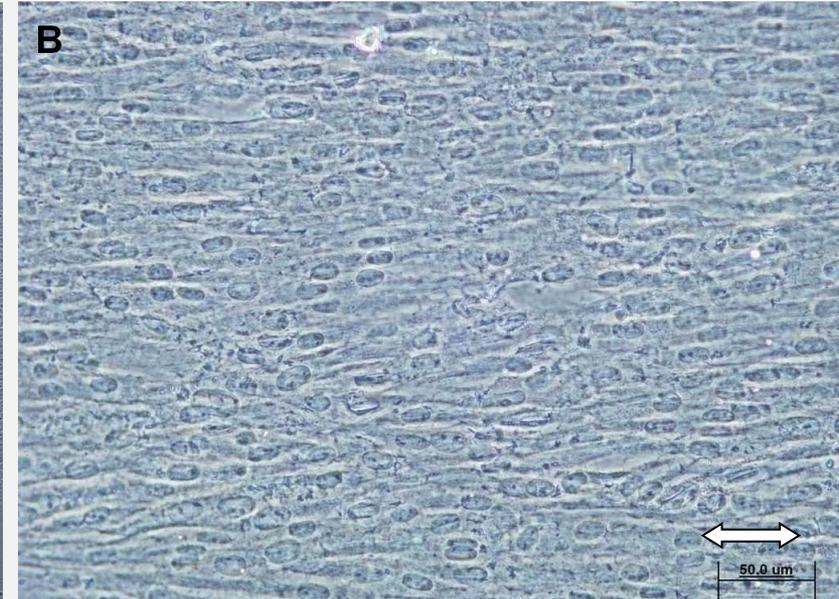


Stretched

Static

1D Stimulation of Smooth Muscle Cells

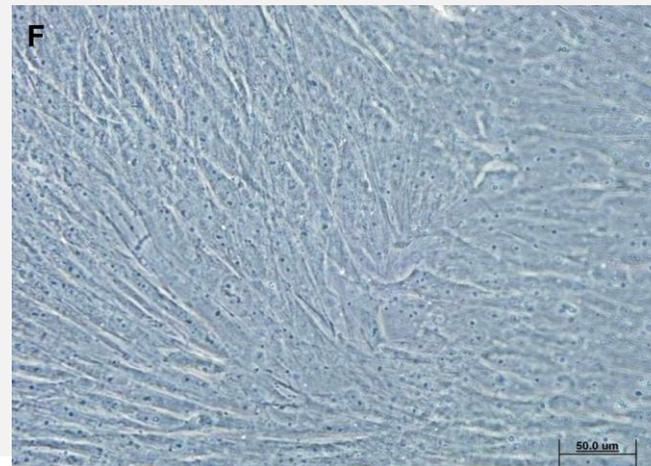
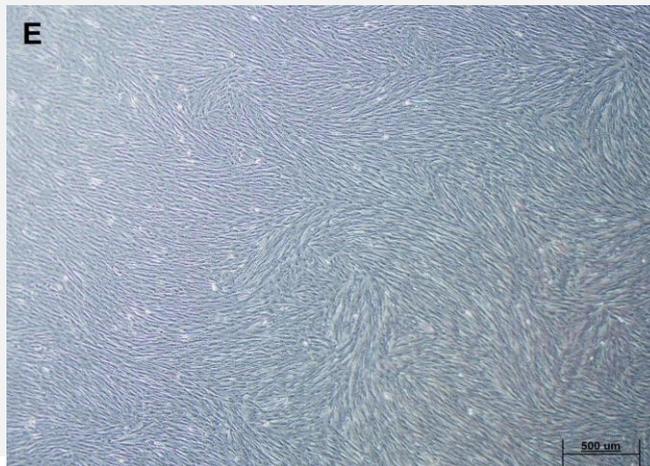
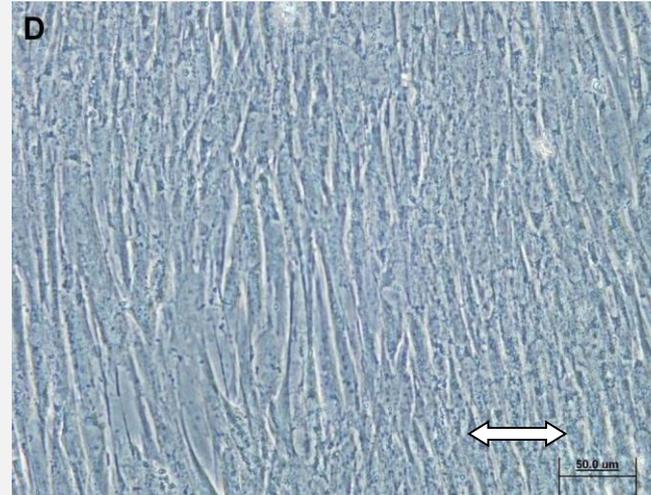
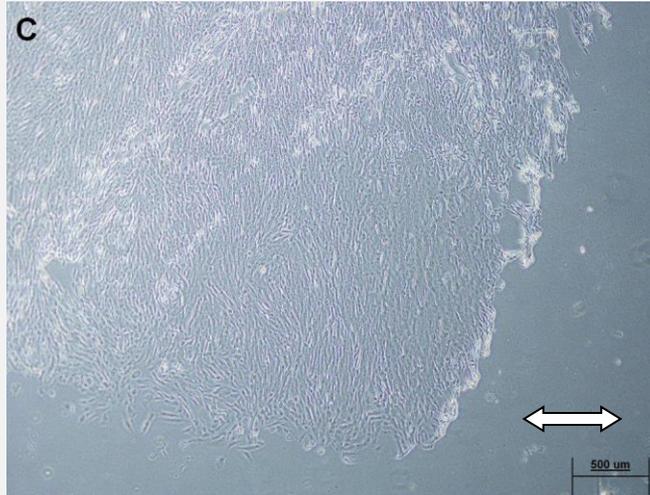
- Oesophageal cycle at 2.5% strain induces alignment parallel to direction of strain, with higher cell survival rates



Static

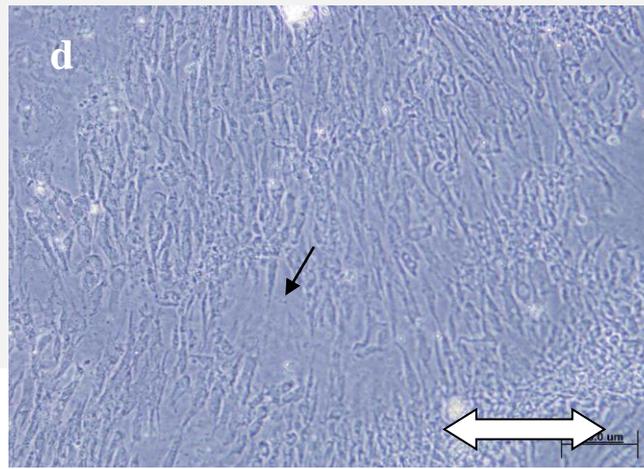
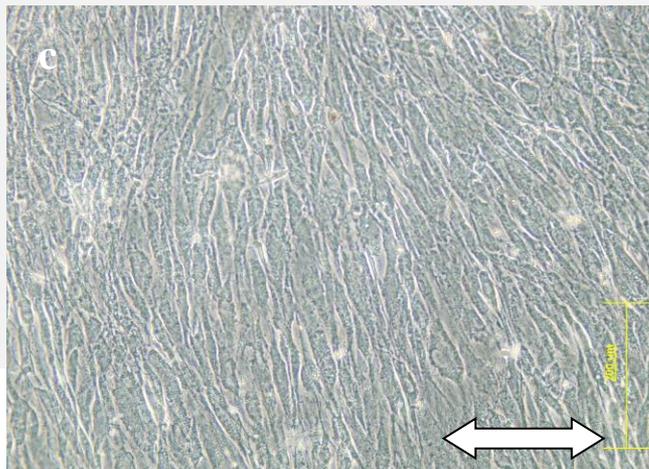
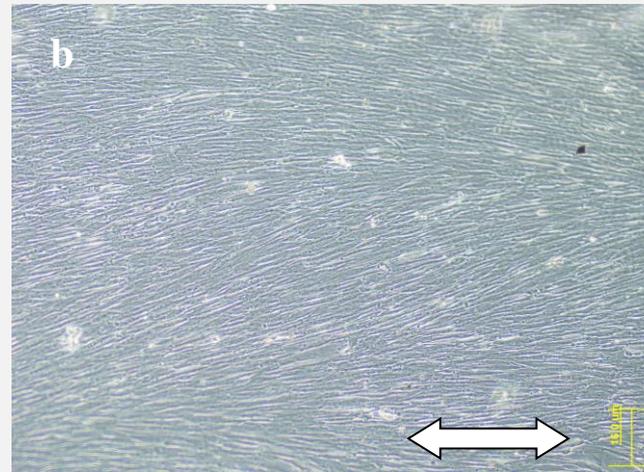
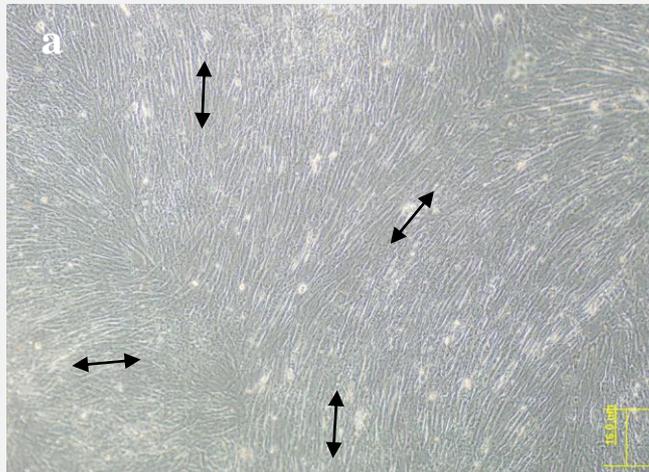
SMC alignment on day 2 of stretching (Light Microscope). A. SMCs at $f = 0.07$ Hz ($\times 40$), B. SMCs at $f = 0.07$ Hz ($\times 400$)
Arrows indicate Direction of stretch. Strain magnitude is 2.5% in all stimulated cells

Cardiovascular Cycle, 2.5% strain



SMC alignment on day 2 of stretching (Light Microscope). C. SMCs at $f = 1$ Hz ($\times 40$), D SMCs at $f = 1$ Hz ($\times 400$), E. Static SMCs ($\times 40$), F. Static SMCs ($\times 400$).

1-D Stimulation – Effect of Strain magnitude



400x magnification

a – static

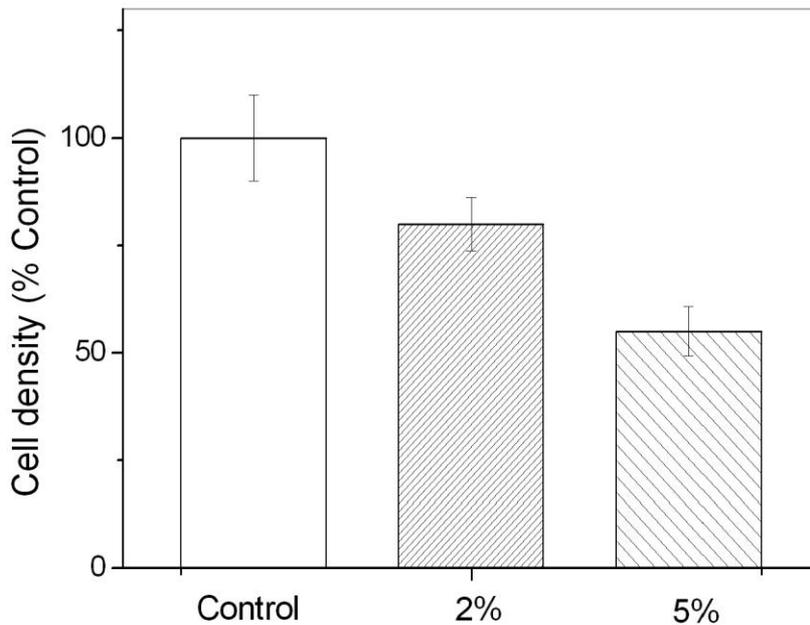
b – 1% strain

c – 5% strain

d – 10% strain

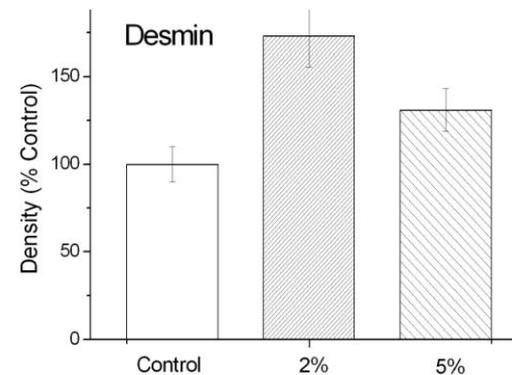
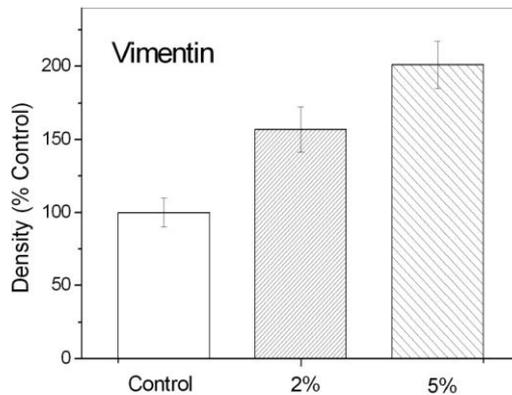
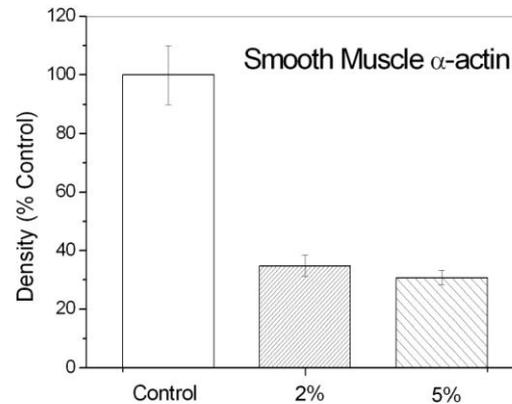
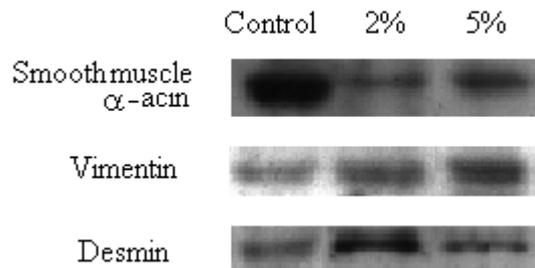
Arrow in d indicates
region of cell loss

Cell proliferation



- CellTiter 96 Proliferation Assay
- Reduction in cell numbers may indicate expression of contractile rather than synthetic phenotype
- Cell loss is more marked at higher strains

Phenotype Expression



- Actin – Structural Protein
- Vimentin – Cell motility
- Desmin – Differentiation marker

Conclusions

- Mechanical stimulation induces differentiation and expression of the contractile phenotype
 - Overstimulation is the product of magnitude and frequency
 - Perpendicular alignment is a stress avoidance response to protect the cytoskeleton and contractile filaments