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Micromechanics in Functionally Distinct Tendons: Tendon Injury and Repair

Screen HRC, Thorpe CT, Shepherd JS, Spiesz EM, Riley GP, Legerlotz K, Birch HL, Clegg PD

School of Engineering and Materials Science

Structure-Function Optimisation

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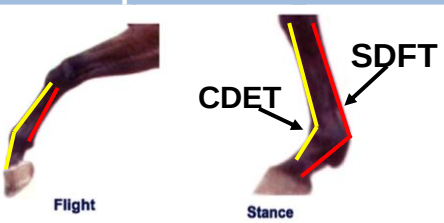
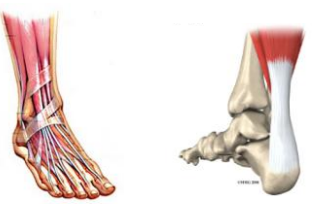




Birch HL (2007) International Journal of Experimental Pathology, 88;4 241-248

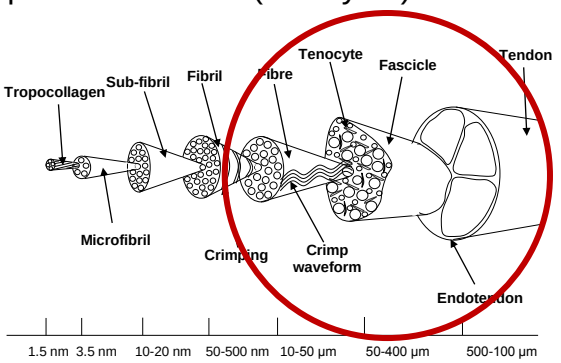
Energy storing tendons	**Positional Tendons**
High strains & elastic recoil in use High incidence of tendinopathy	Low strains and less elastic Efficient strain transfer from muscle-bone

Different Hierarchical Mechanical Properties

Tendon structural mechanics are optimised to suit function

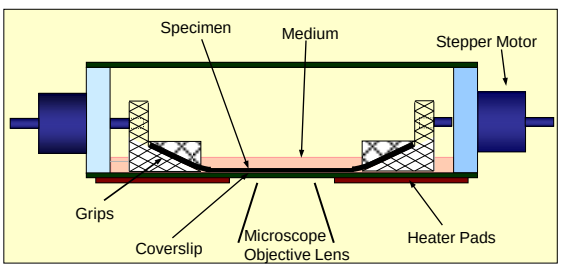
Tendon Structure

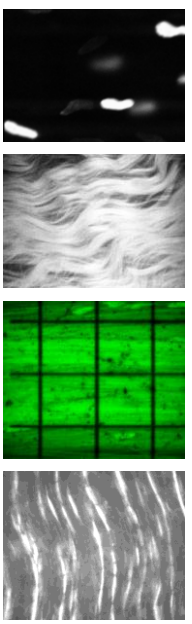
- Fibre composite material
  - Multiple hierarchical levels of collagen
  - Proteoglycanous matrix binding
  - Interspersed with cells (tenocytes)



In Situ Analysis Techniques

- Custom designed rig for location on confocal microscope
- Enables tensile / compressive loading of viable tissue samples
- Use range of matrix & cell stains to visualise matrix components during straining





Screen et al. (2003) *Biorheol.* 40, 361-8

Screen et al. (2004) *J. Eng. Med.* 218, 109-19

Cheng & Screen (2007) *J. Mat. Sci* 21; 8957-65

Micromechanics & Energy Storing Tendons

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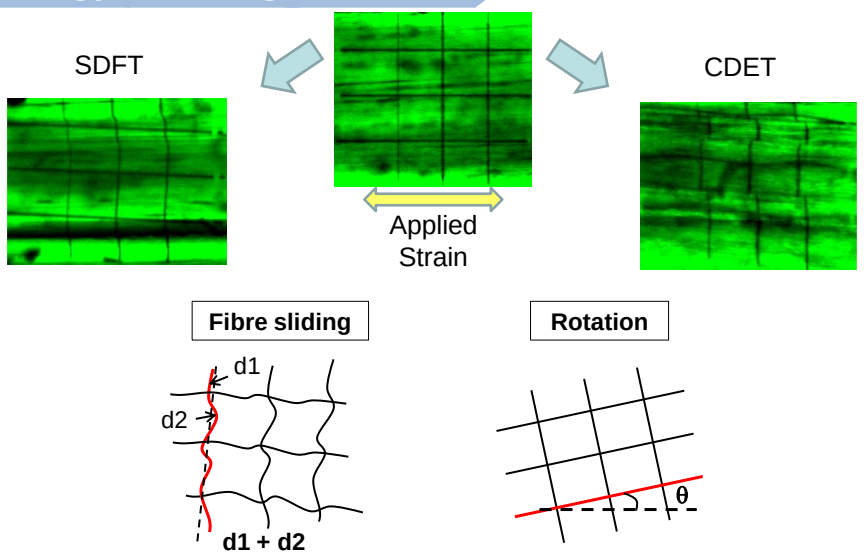

Thorpe et al. (2013) *Acta Biomaterialia* 9:8; 7948-56

Fibre sliding & Rotation

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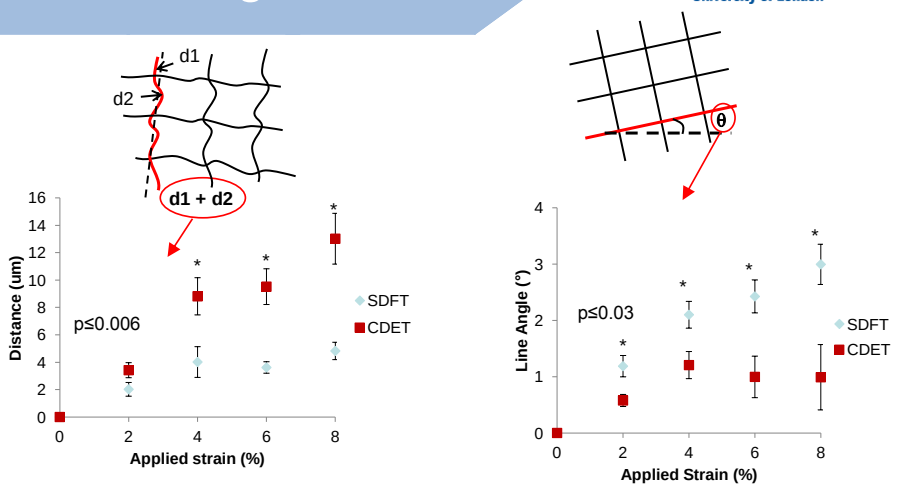

Significantly more fibre sliding in positional tendons

Significantly more fascicle rotation in energy storing tendons

Thorpe et al. (2013) *Acta Biomaterialia* 9:8; 7948-56

Micromechanics

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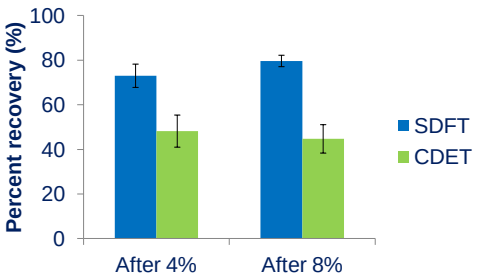
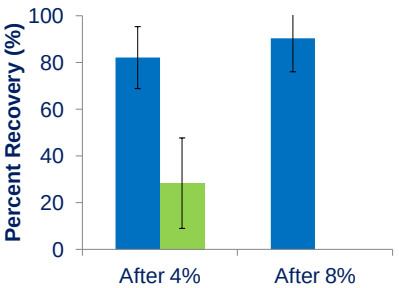
Recovery

Fibre Sliding



Fascicle Rotation



Significantly better recovery from loading in energy storing flexor fascicles

Thorpe et al. (2013) *Acta Biomaterialia* 9:8; 7948-56

Summary

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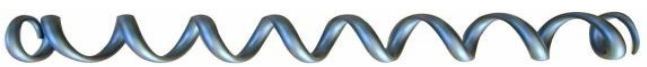
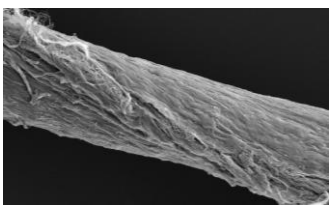
SDFT	CDET
• Extension = sample rotation	• Extension = fibre sliding
• Good recovery & elasticity	• Poor recovery & less elastic

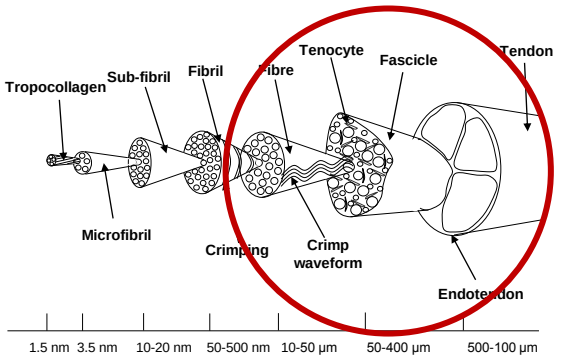


Fascicles in energy storing SDFTs have helical component which enables efficient extension and recoil



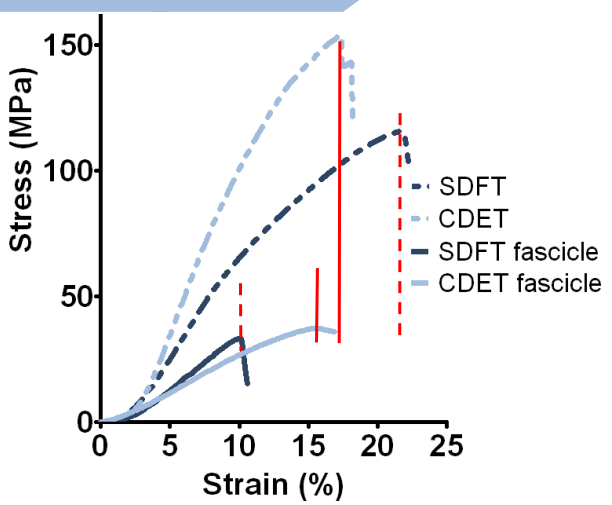
Thorpe et al. (2013) *Acta Biomaterialia* 9:8; 7948-56

Fascicle / Tendon Mechanics



Fascicle contribution to tendon mechanics?

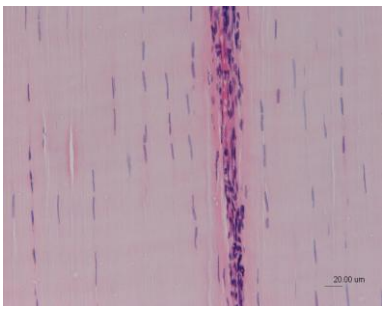
Whole tendon mechanics



Typical stress strain curves for tendons & fascicles

Thorpe CT et al. (2012) *J Roy Soc Int* 9:76; 3108-17

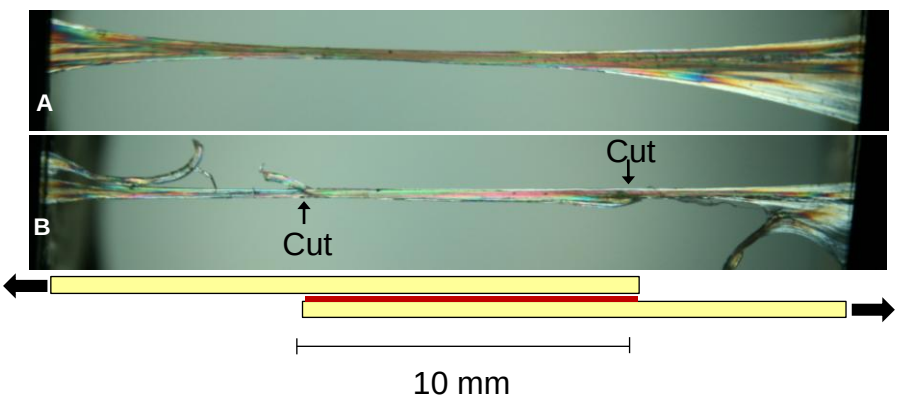
Interfascicular matrix



Building indications of fascicle ends

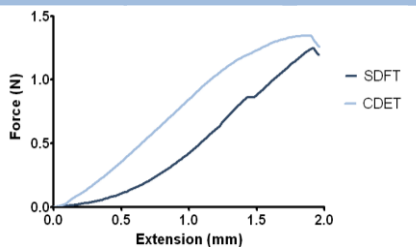
Fibre composite mechanics at the fascicle level?

Interfascicular mechanics

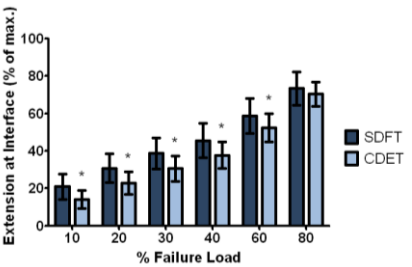


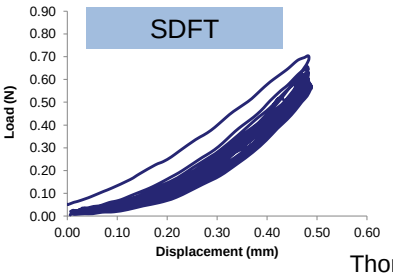
Thorpe CT et al. (2012) *J Roy Soc Int* 9:76; 3108-17

Interfascicular mechanics



Typical force extension curves





- At and below 60% of failure load, extension at interface is significantly greater ($p<0.001$) in the SDFT

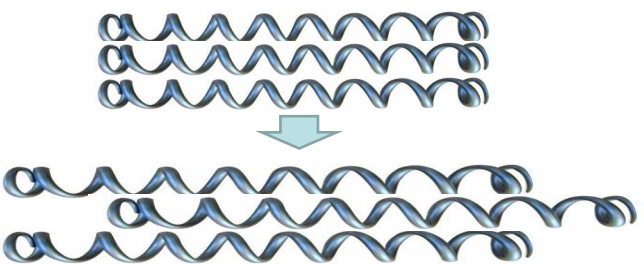
Thorpe CT et al. (2012) *J Roy Soc Int* 9:76; 3108-17

Extension mechanisms - SDFT

More springy, elastic fascicles in energy storing tendons

Extend and recover using a helical mechanism

Slide relative to each other within the whole loaded tendon to enable large strains



Ageing & Energy Storing Tendon Mechanics

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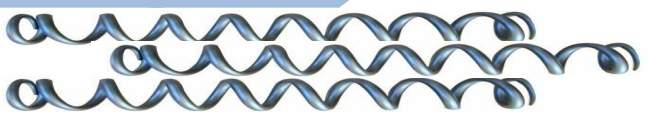


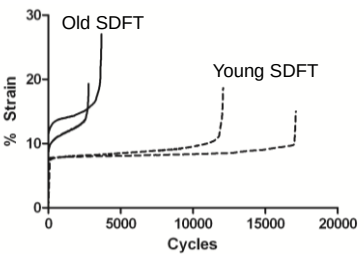
Tightness of the helix reduces

Recoverability of the helix reduces

Capacity for fascicle sliding reduces

Thorpe CT et al. (2014) *J Roy Soc Int* 11:92; 20131058
Thorpe CT et al. (2013) *Eur Cell Mat* 8:25; 48-60

Correlates with reduced fatigue resistance:



Tendinopathy

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- Why are some individuals prone to tendinopathy in energy storing tendons?
- What happens under cyclic fatigue loading?
  - Micromechanics
  - Cell response

SDFT Focus

Fatigue Loading - Micromechanics

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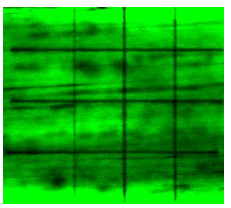
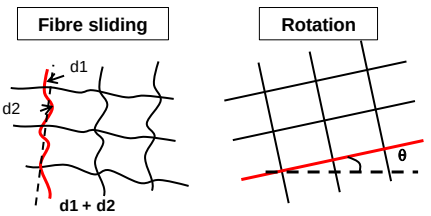
SDFT Fascicles

Young (3-6yrs); Old (18-20yrs)

- Subject half to fatigue loading:
  - Cyclic creep: 60% UTS; 1800 cycles





Thorpe et al. (2014) *Acta Biomat 10:7; 3217-24*

Fatigue Loading - Micromechanics

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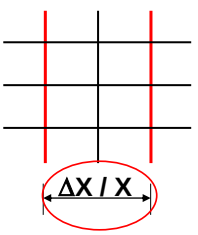

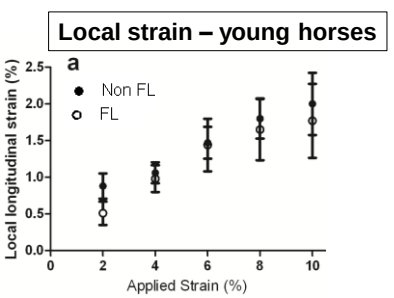

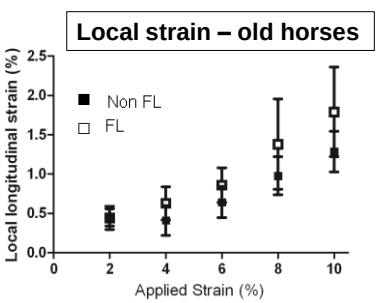

Thorpe et al. (2014) *Acta Biomat 10:7; 3217-24*

Fatigue Loading - Micromechanics

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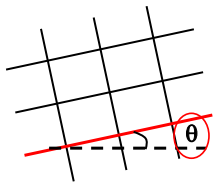
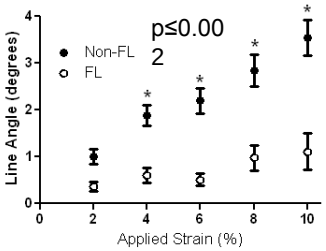
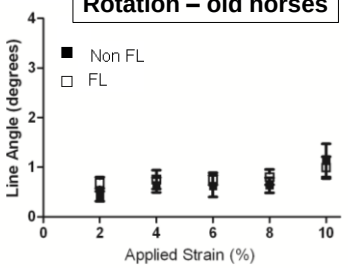


Rotation – young horses



Rotation – old horses



Thorpe et al. (2014) *Acta Biomat 10:7; 3217-24*

Fatigue Loading - Micromechanics

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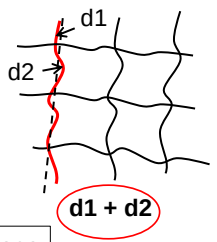
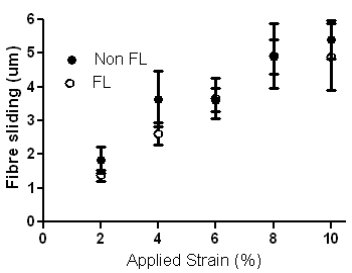
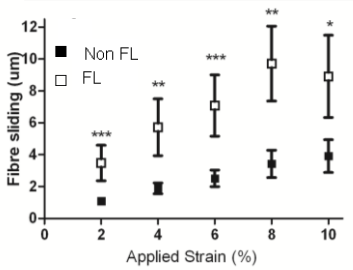


Fibre sliding – young horses

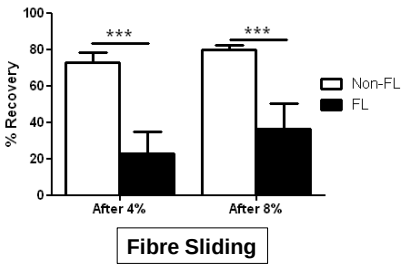


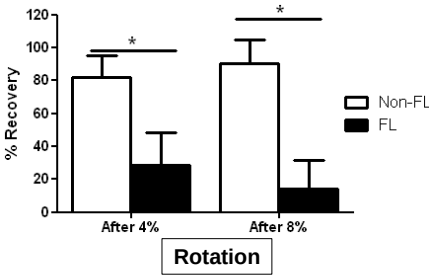
Fibre sliding – old horses



Thorpe et al. (2014) *Acta Biomat 10:7; 3217-24*

Ability to recoil – Young SDFT





Thorpe et al. (2014) *Acta Biomat 10:7; 3217-24*

Fatigue Loading & Ageing

SDFT (young)

- Grid rotation enables spring like high extensions

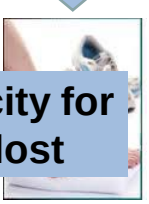
SDFT (old)

- Spring coil reduced and recovery of samples limited

Fatigue Load Sample

- Resists damage using spring mechanism
- Starts to reduce spring coiling

- No mechanism to resist loading = resorts to fibre sliding



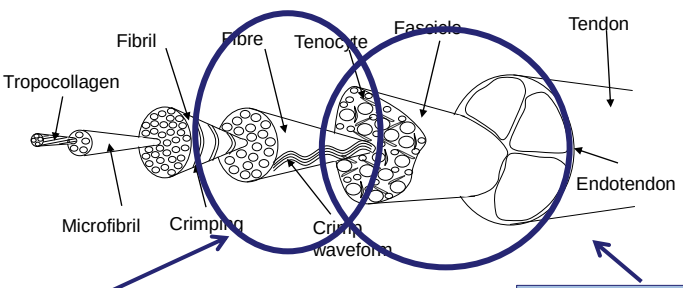
As the spring is lost, capacity for recoil and recovery is lost

Tendinopathy

- Why are some individuals prone to tendinopathy in energy storing tendons?
- What happens under cyclic fatigue loading?
  - Micromechanics
  - Cell response

SDFT Focus

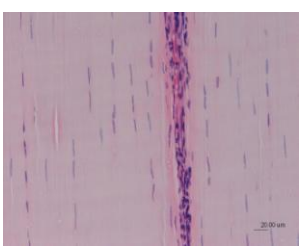
Tendon Micromechanics



Positional tendons rely on fibre sliding

Fascicular Cells = high shear low tension

Inter-Fascicular Cells = low shear some tension



Energy storing tendons rely on helix uncoiling & fascicle sliding

Fascicular Cells = low shear some tension

Inter-Fascicular Cells = high shear low tension

Cellular Response to Fatigue Loading

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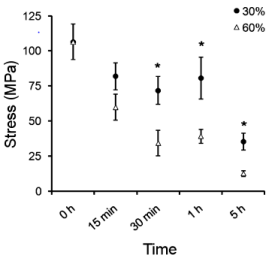
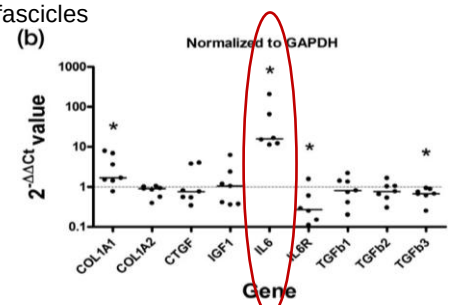
Cyclic loading of fascicles

1800 Cycles: from 0-60% of UTS

Characterising damage: bovine positional fascicles





Significant upregulation of IL-6 with loading

Legerlotz et al (2013) *Scand J Med Sci Sport.* **23:1**; 31-37.

Cellular Response to Fatigue Loading

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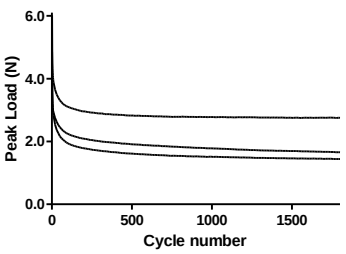
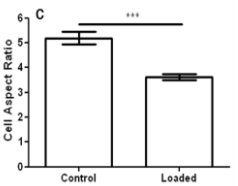
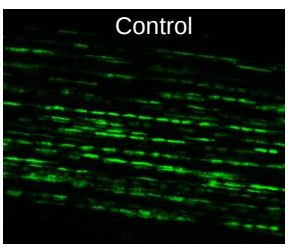
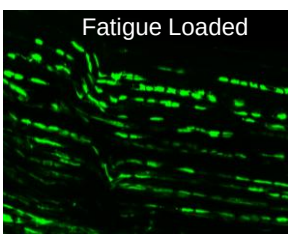
1800 Cycles: from 0 to 60% of UTS

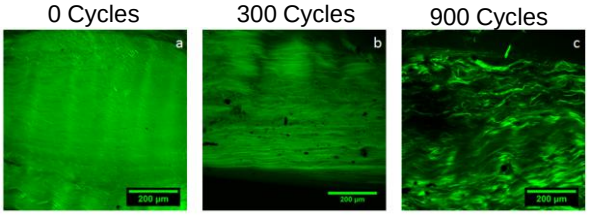








0 Cycles | 300 Cycles | 900 Cycles



Bovine Tissue: Shepherd et al. (2014) JMBBM *in press*

Thorpe et al. (2014) Submitted to SJMSS

Cellular response to Fatigue Loading

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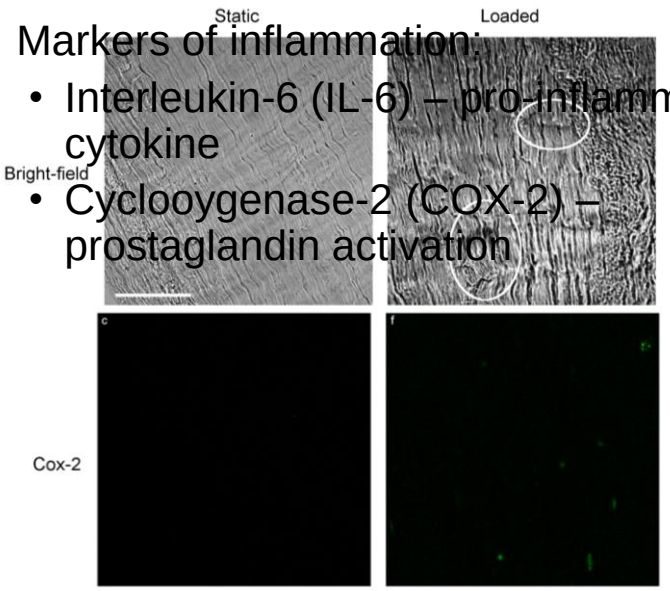
- Markers of inflammation:
  - Interleukin-6 (IL-6) – pro-inflammatory cytokine
  - Cyclooygenase-2 (COX-2) – prostaglandin activation



Increase in IL-6 and COX-2 with loading

Response greater in the IFM

Thorpe et al. (2014) Submitted to SJMSS

Cell response to cyclic loading - degradation

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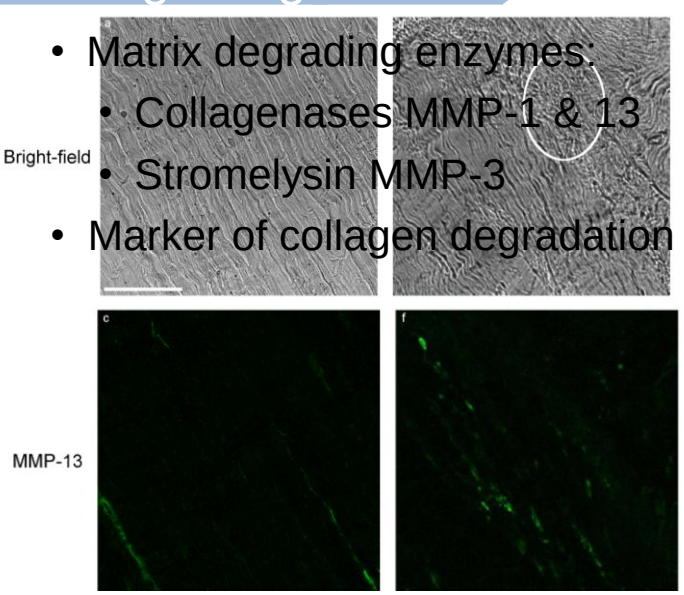
- Matrix degrading enzymes:
  - Collagenases MMP-1 & 13
  - Stromelysin MMP-3
- Marker of collagen degradation



Diffuse increased staining for MMP-13 with loading

Thorpe et al. (2014) Submitted to SJMSS

Cell response to cyclic loading - degradation

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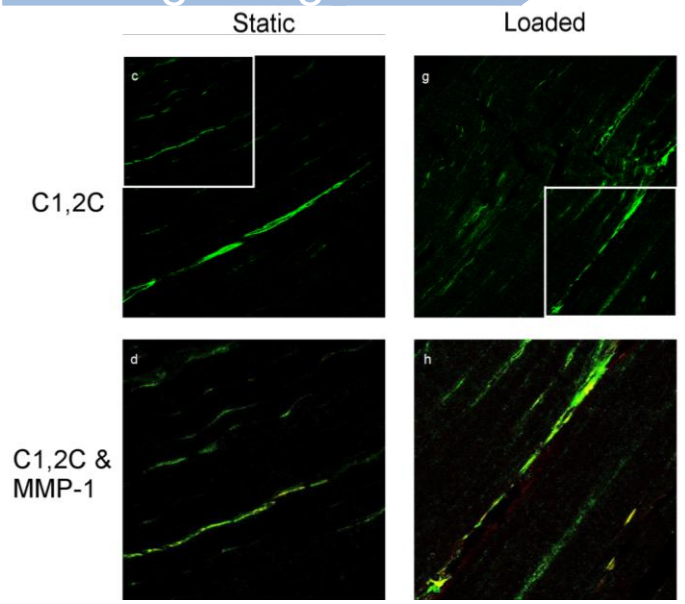

Increased MMP-1 with loading

MMP-1 also in statically loaded samples: co-localised to C1-2C

IFM localised without load; Throughout matrix with load

IFM shows more turnover?

Thorpe et al. (2014) Submitted to SJMSS

Cell response to cyclic loading - summary

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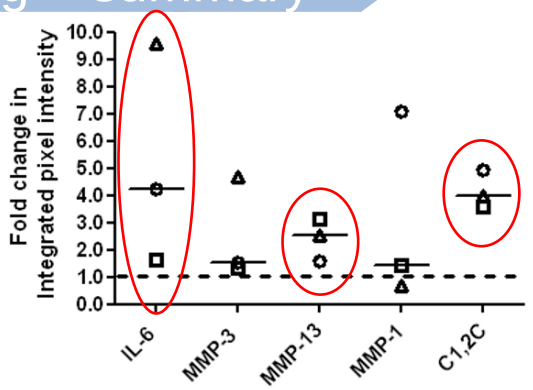

Indications of continual IFM turnover: MMP-1 and C1-2C in healthy tissue

Overload

Increase inflammatory markers with overload; specifically in IFM

Increased collagen degradation activity with loading – throughout matrix

What's Next?

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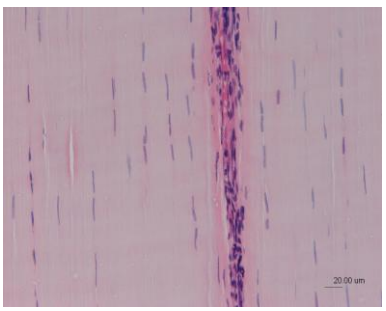
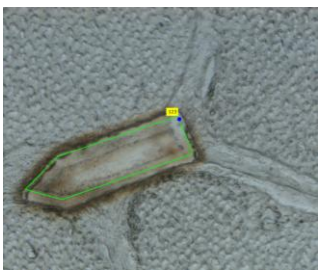
IFM turnover important for health?
IFM cells initial responders to overload?
What is in the IFM – does it assist energy storing tendon function?



Laser capture microdissection to isolate regions of FM & IFM



- Proteomics
- Histochemistry
- Cell Phenotype

→ Developing Tendinopathy

wellcome trust
Arthritis Research UK
EPSRC Pioneering research and skills
HBLB
The Royal Academy of Engineering
BBSRC bioscience for the future

Contributing Lab Members: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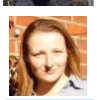
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