"Effect of cyclic tensile load on the regulation of the expression of matrix metalloproteases (MMPs -1, -3) and structural components in synovial cells."

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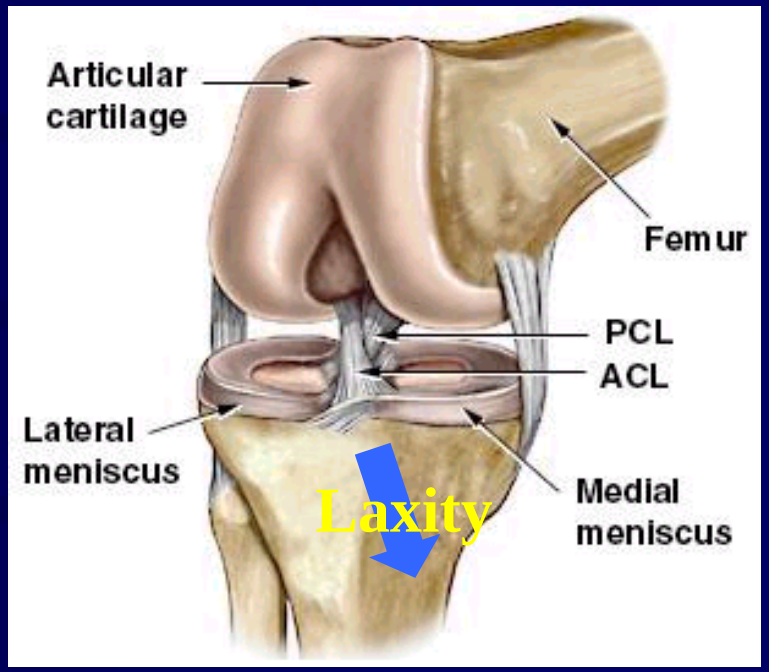
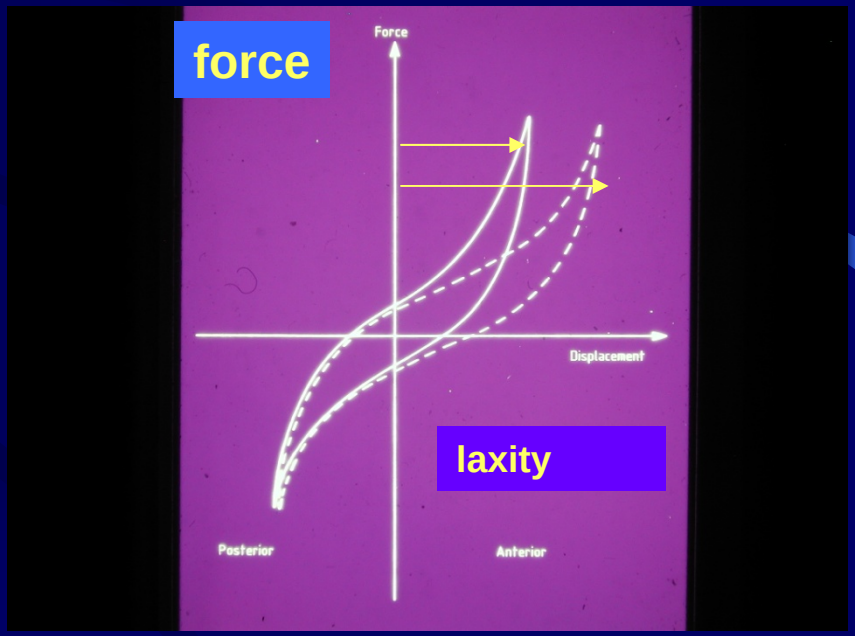
*E*ngineering of Anterior cruciate ligament:

A review of aspects of the process of tissue induction in the canine, the human and laboratory models

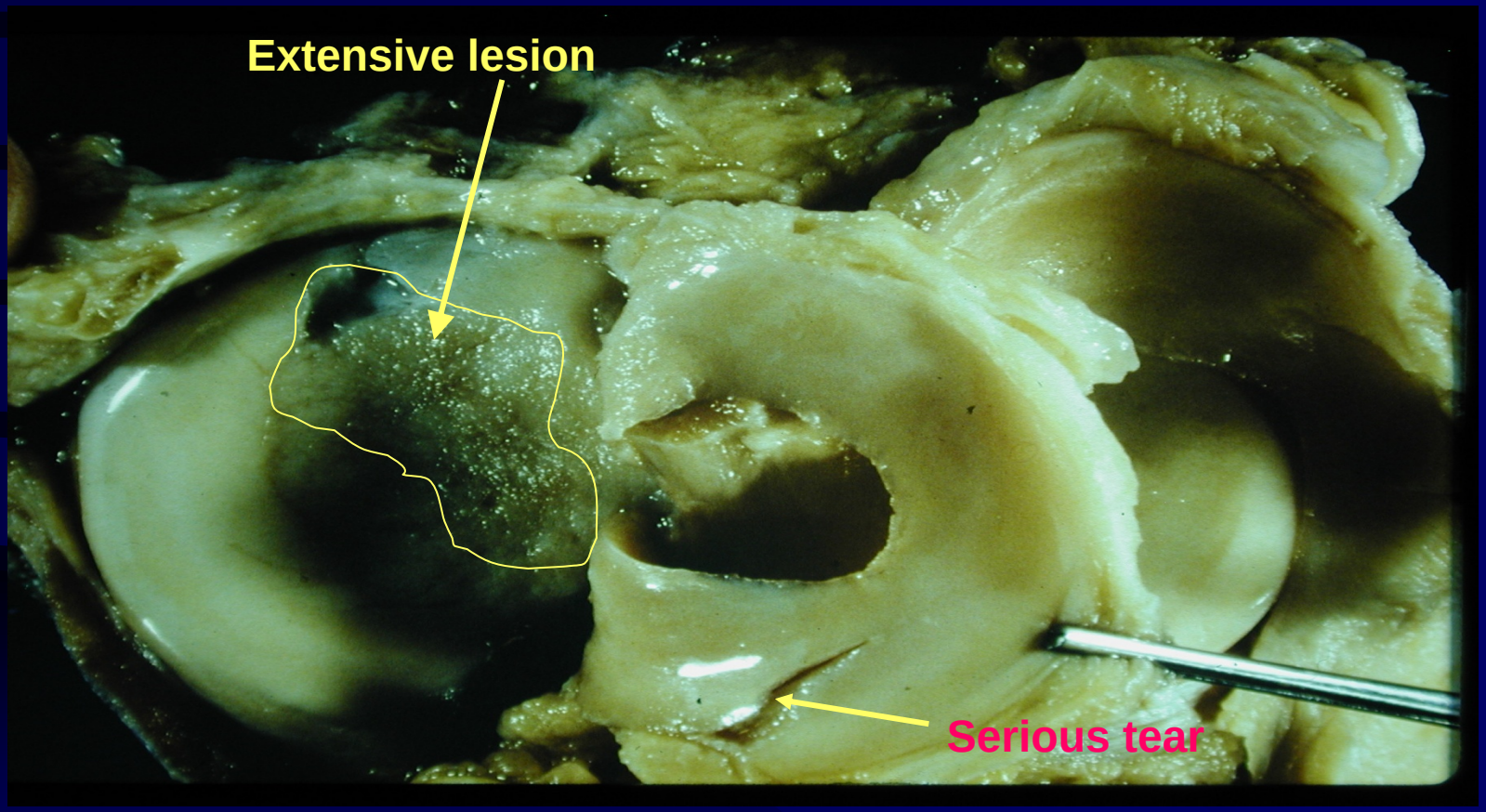
Function of the ACL

- The ACL primarily functions as a constraint on the anterior movement of the tibia with respect to the femur.
- When the ACL is ruptured this constraint disappears and the knee becomes lax.
- As Joint laxity increases the joint can become unstable



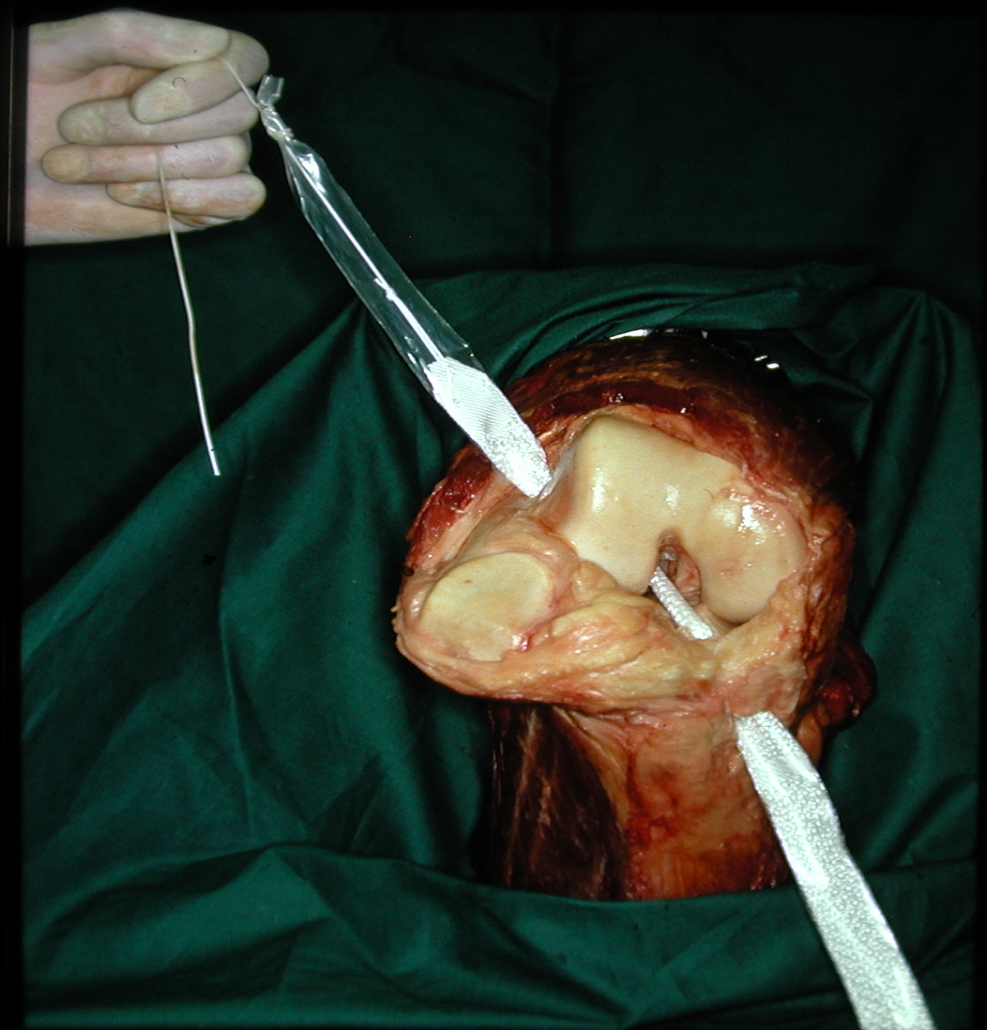


- With increasing laxity the back movement of the femur may have a shearing action on the meniscus causing damage to its posterior horn.



- OA lesions frequently follow injuries to the menisci

Because of the poor healing of the ACL, its reconstruction remains a viable way to restore normal knee function



ACL Reconstruction

- At present mostly done with autogenous tissue
- Synthetic devices were used in the past but now are less popular,

  however these might still have a place in the future: Autogenous reconstructions are invasive
- Tissue engineering of ligaments is an emerging interest.

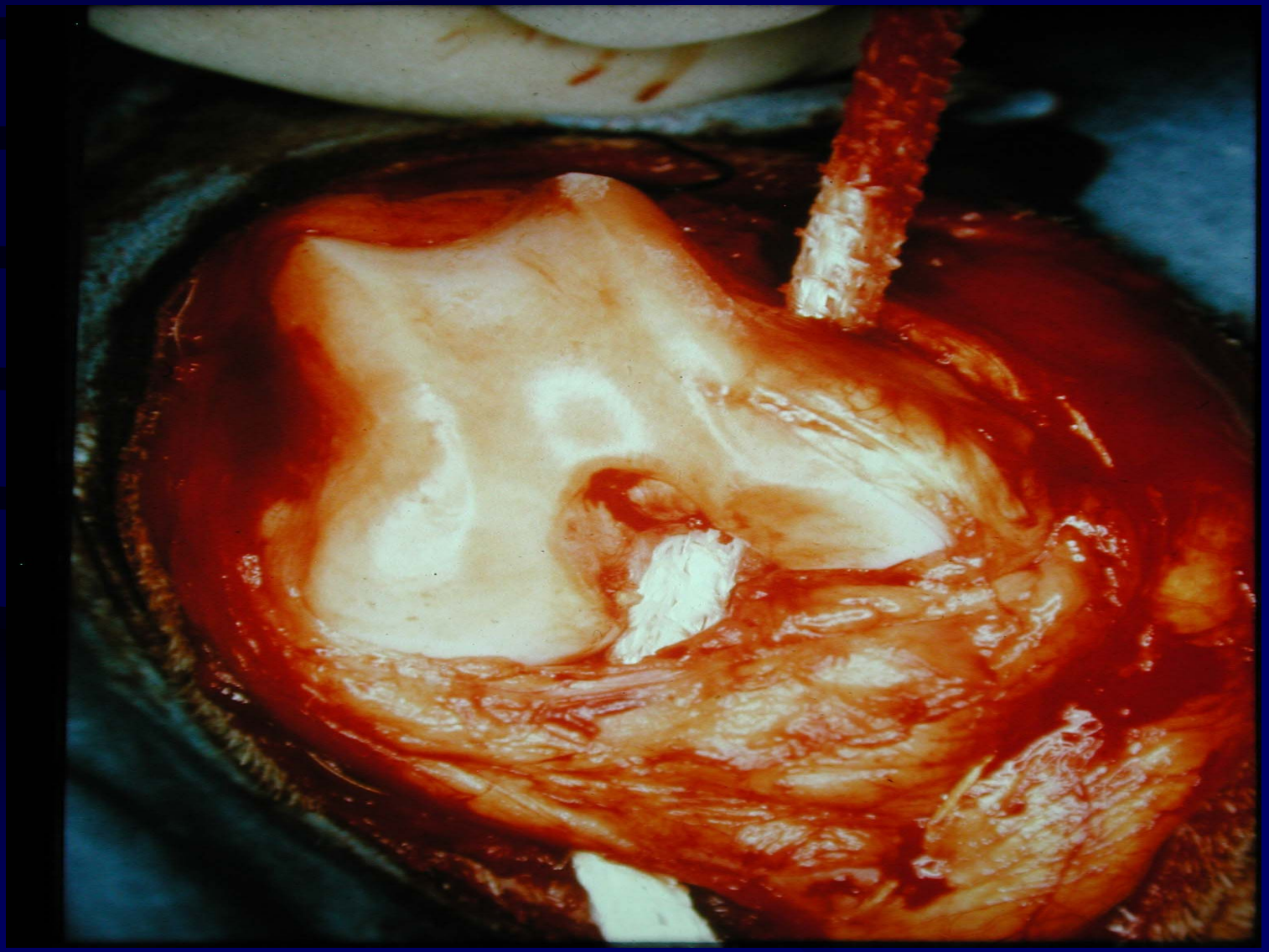
- Autografts and Allografts, after implantation, are subjected to histological and biochemical transformation

→ Their original characteristics are lost and those of ligaments are acquired

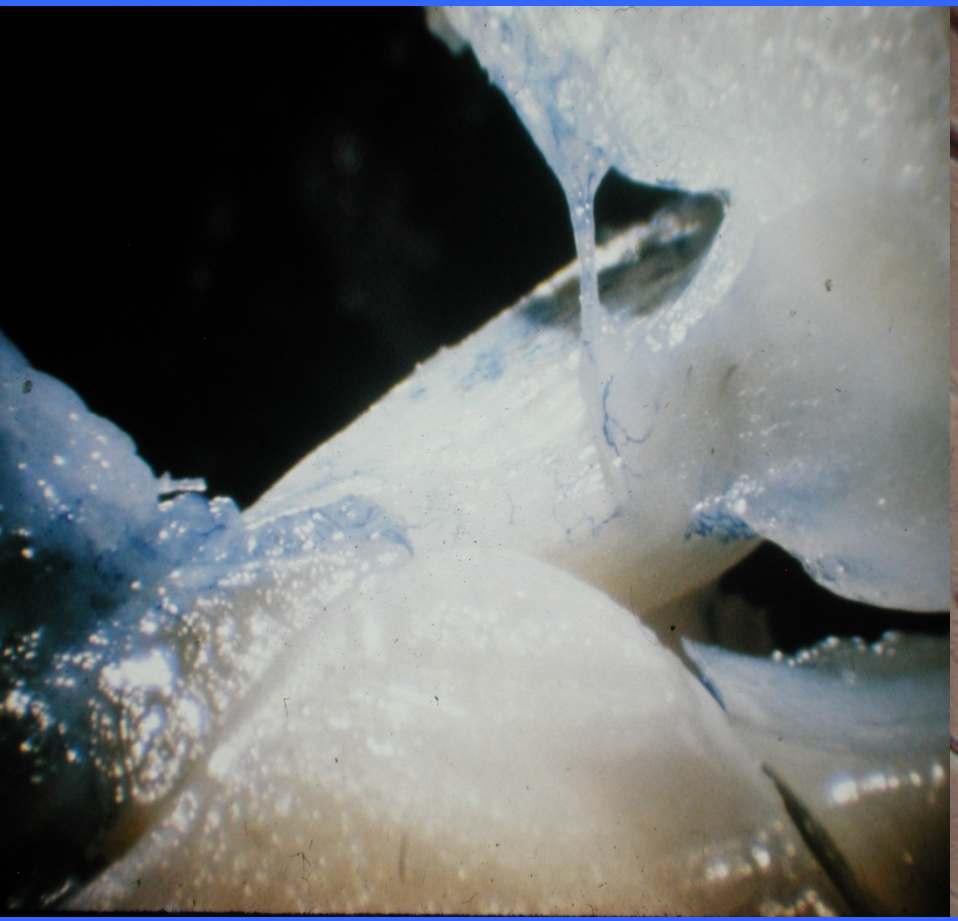
- Therefore host cells appear to repopulate the grafts regardless of the source of the grafted material

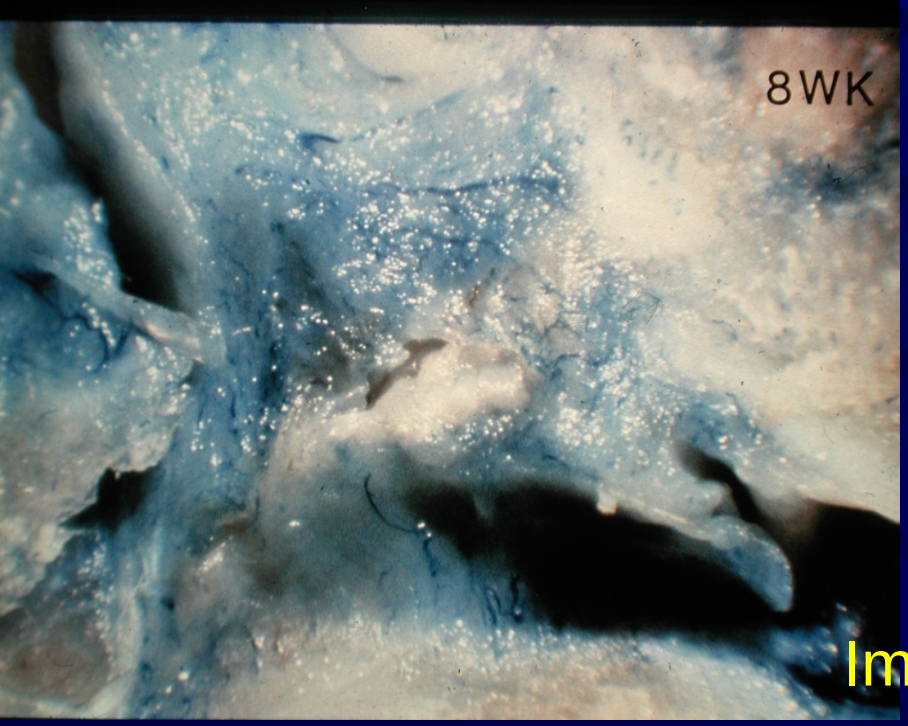
In vivo studies

Early studies in both canine and human models investigated the process of tissue induction around ligament scaffolds. (Fujikawa *et al* 1989,1993)

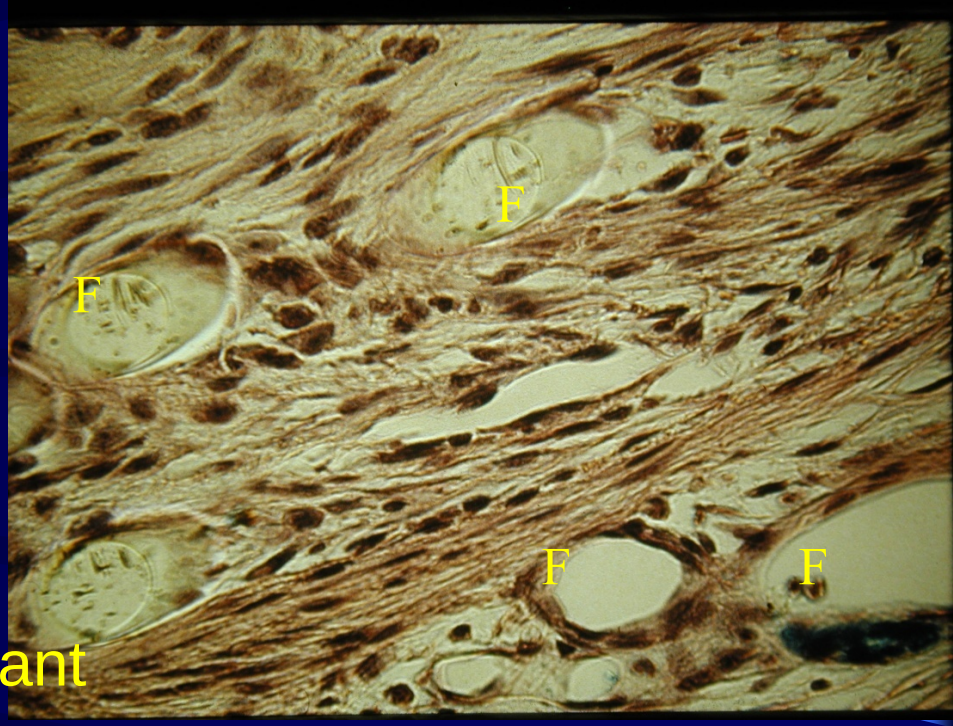


Morphology and histology of the normal canine ACL

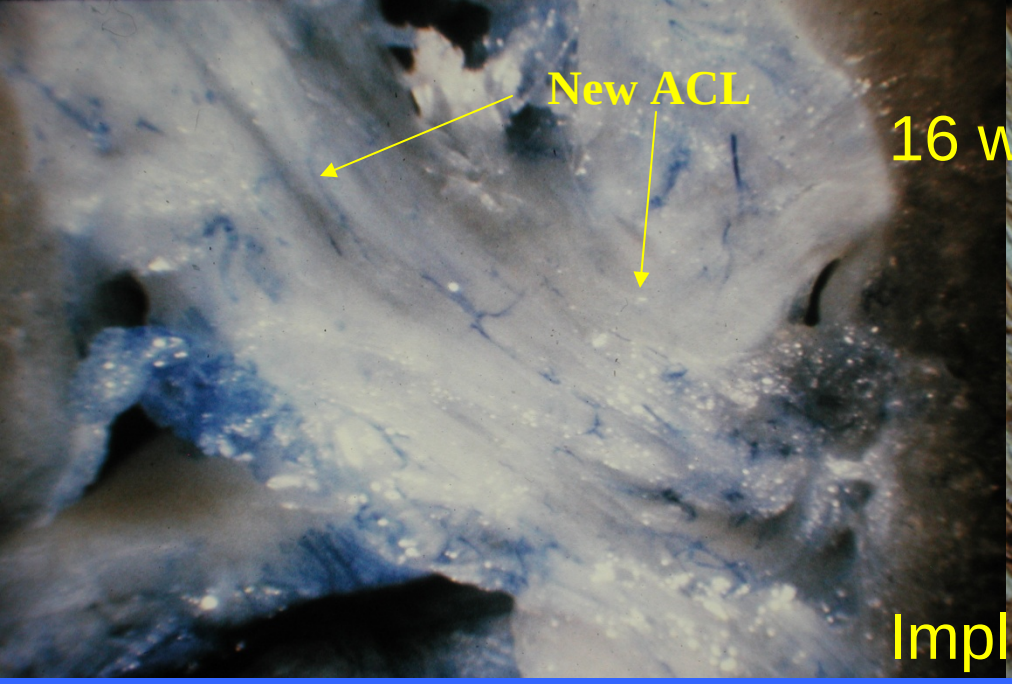




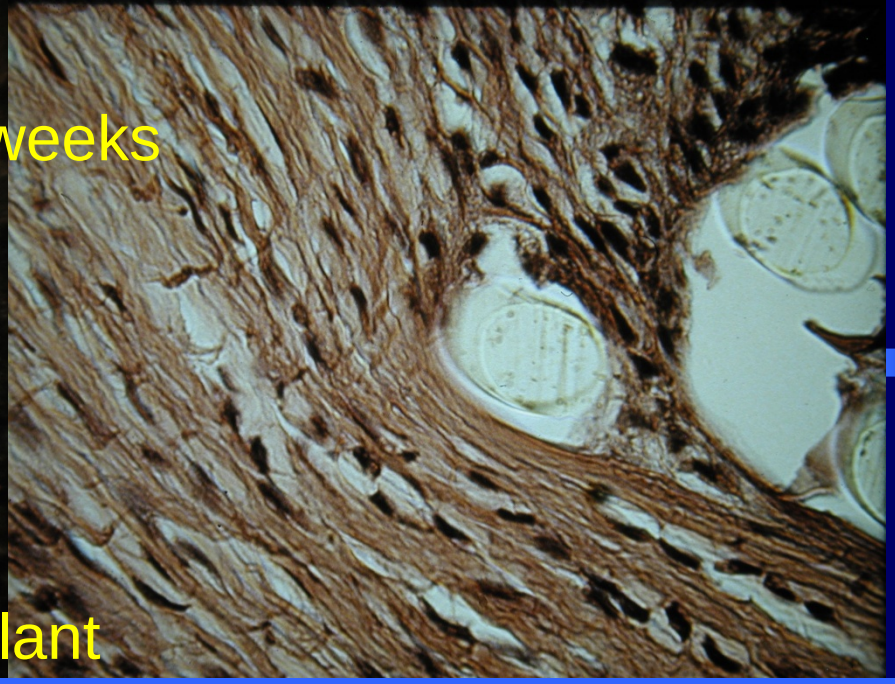
Implant



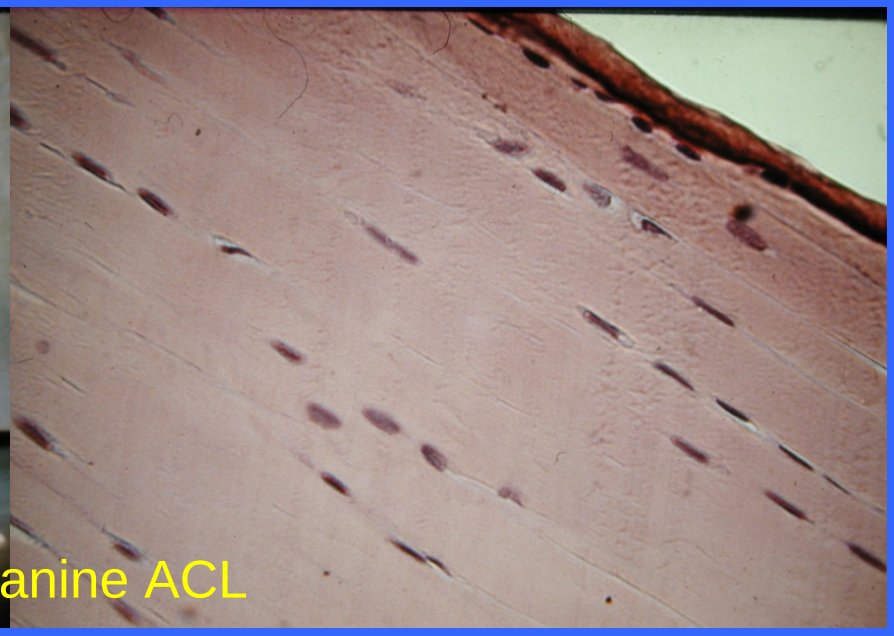
Normal Canine ACL



16 weeks

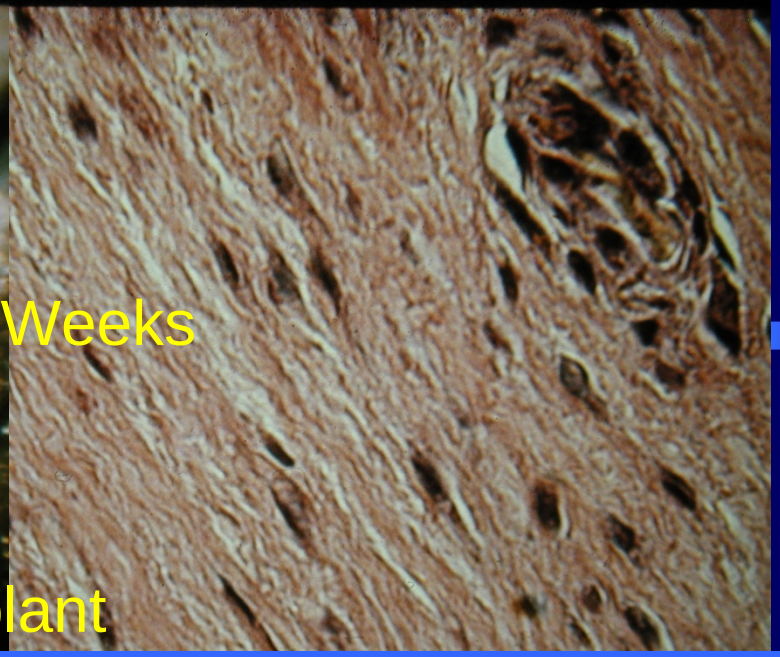


Implant

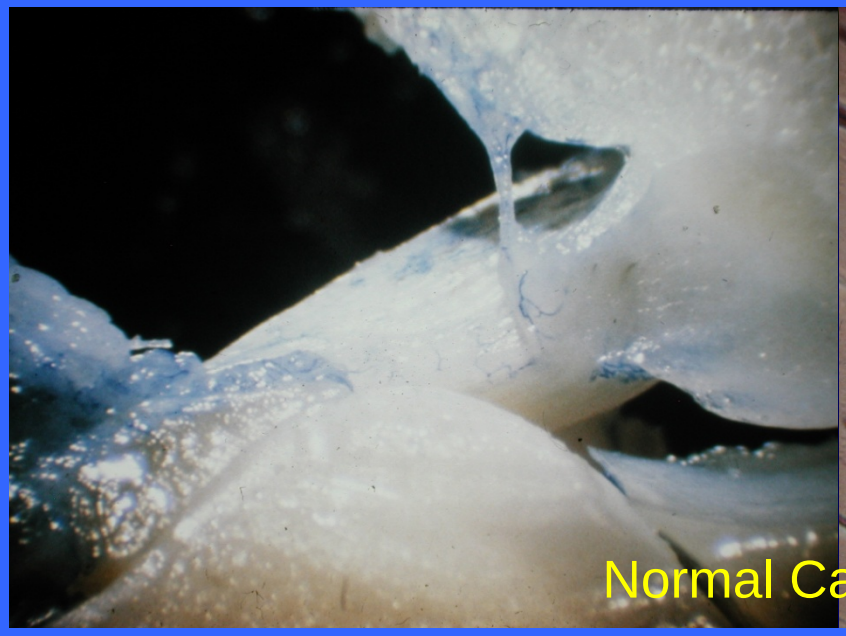


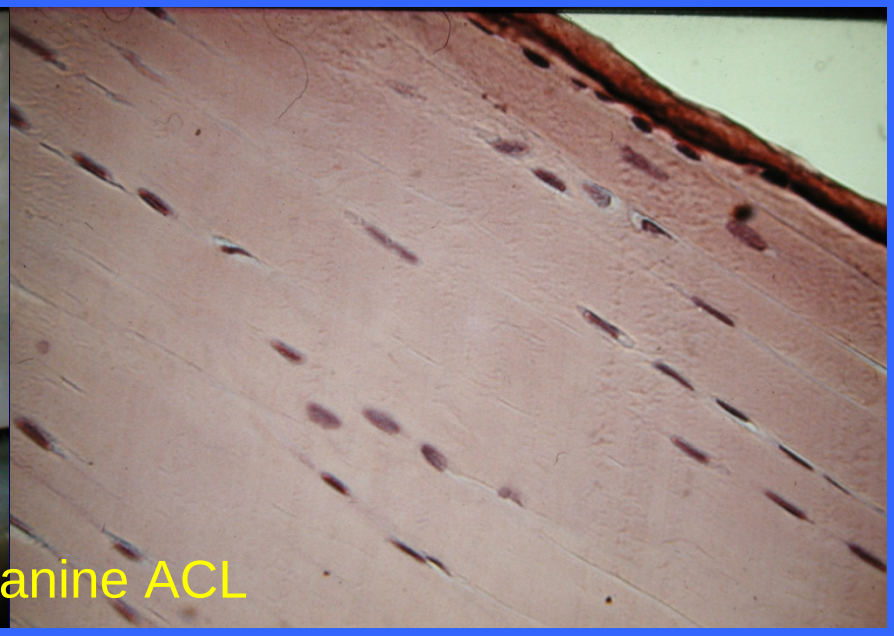
Normal Canine ACL

24 Weeks

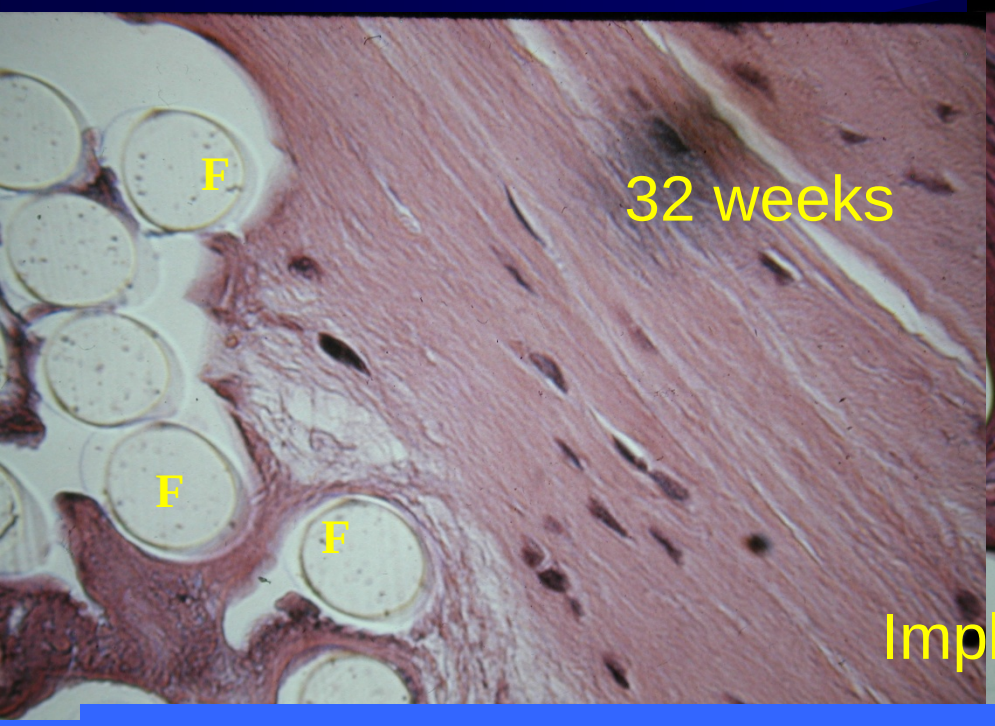


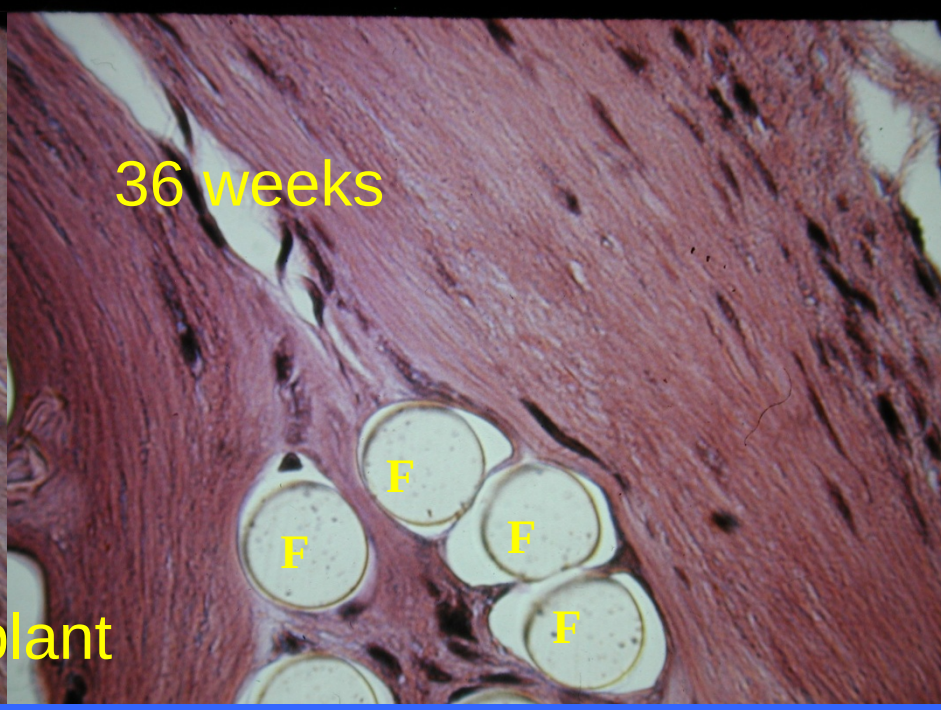
Implant



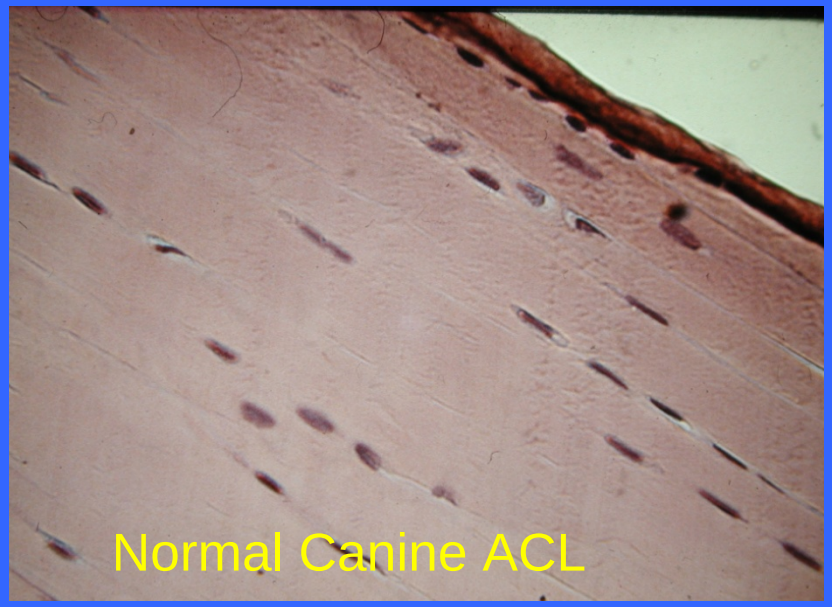


Normal Canine ACL

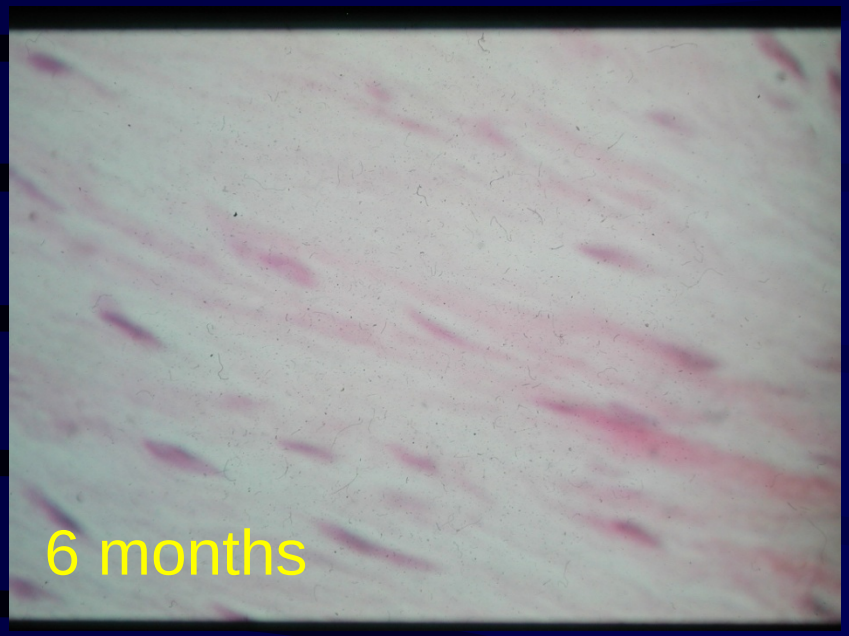


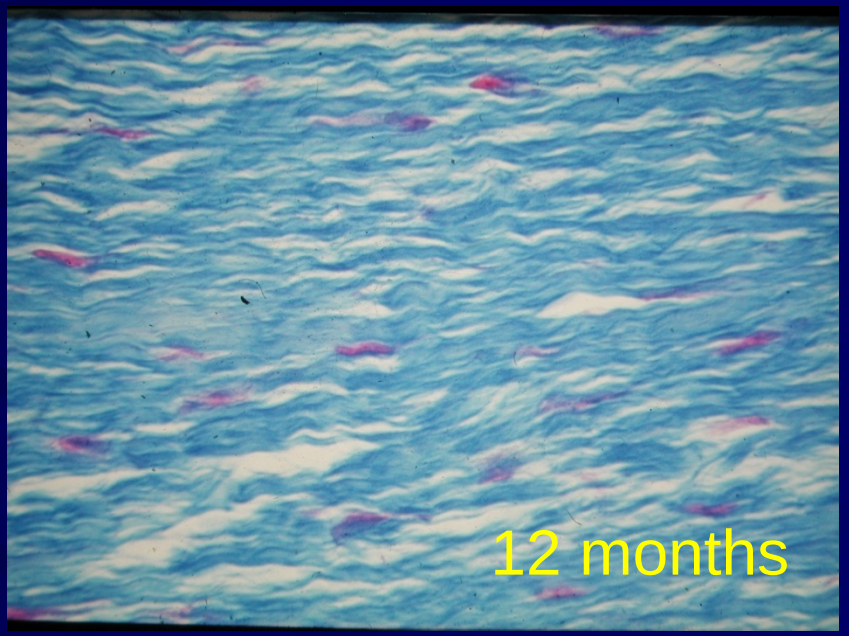


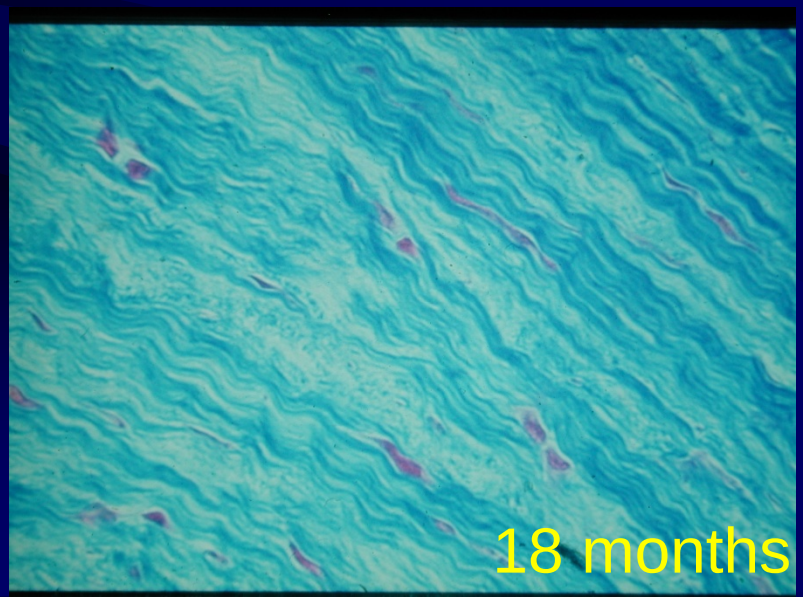
Implant



Human model

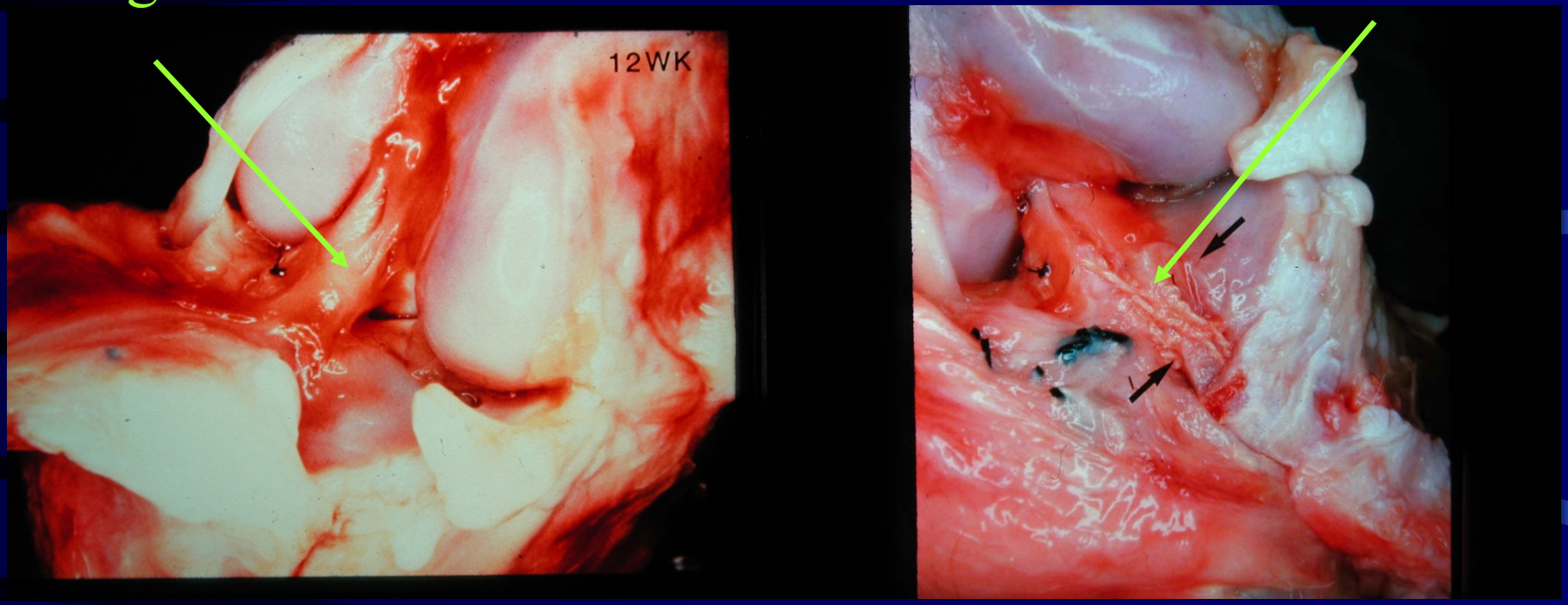






taut ligament

slack ligament



This process of tissue induction and modelling was observed in taut implants, but not in loose ones. In the latter tissue atrophied and remained random and hyper-cellular.

In situ Ligament Engineering

Thus early studies in both canine and human models revealed the involvement of synovial tissue and the importance of tensile strains for the occurrence of cell differentiation and tissue remodelling

Limitations of *in vivo* studies

- Whether there is a threshold for *strain?*
- Effect of the periods of loading on the tissue induction process

Both are relevant to success of the reconstruction and post operative rehabilitation

Both of the above issues are difficult to study *in vivo*

In Vitro investigations

Despite their limitations their value is in the ability to control variables :

(i) strain amplitude,

(ii) frequency,

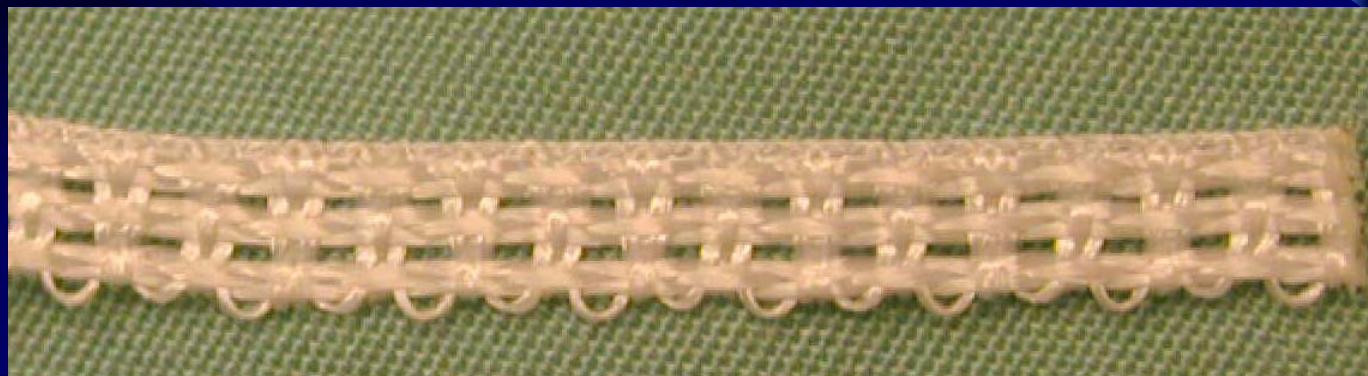
(iii) periods of application (exercise)

Which cells?

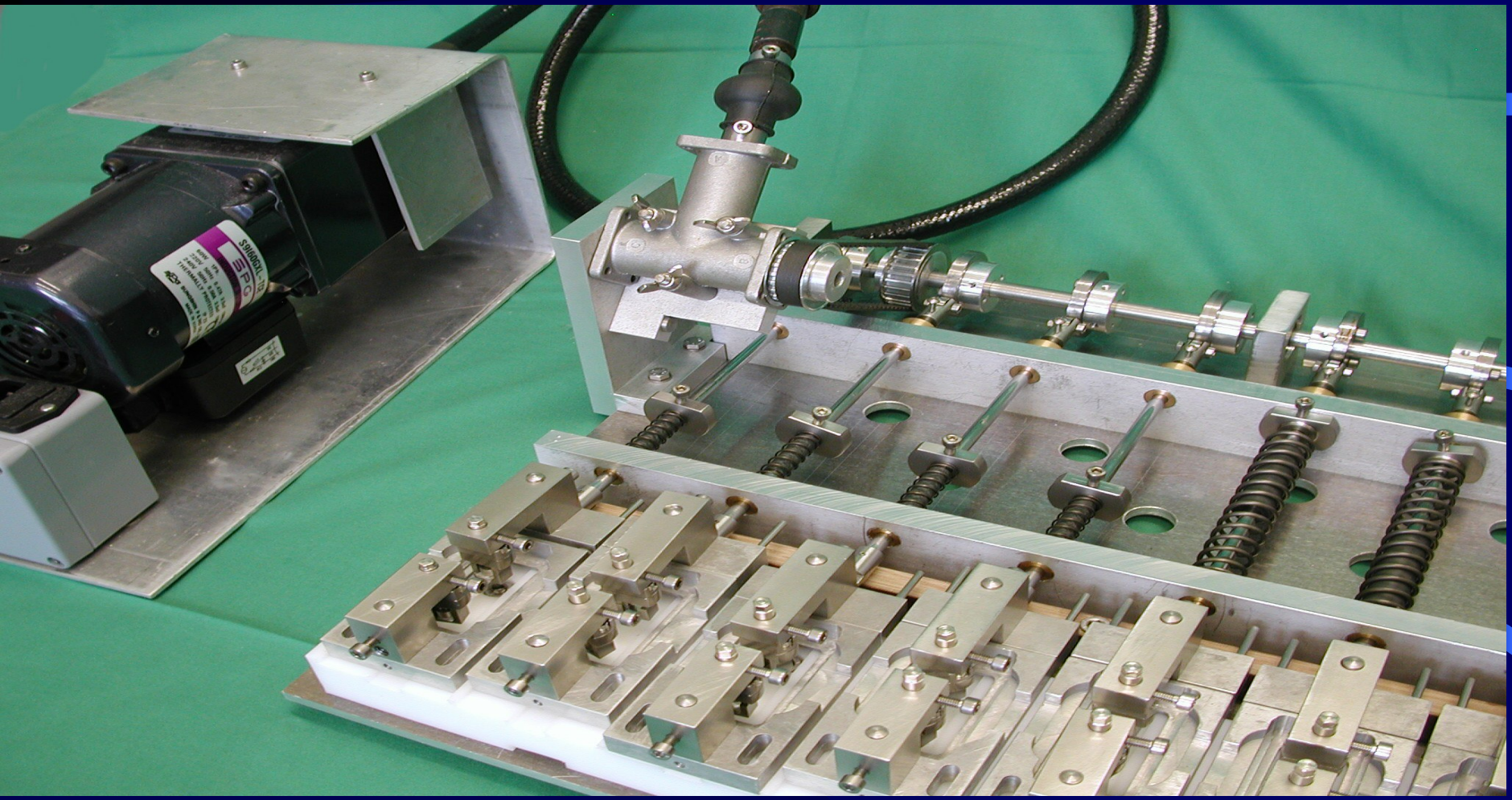
- *Synovial cells,* from bovine Metatarsophalangeal joints

Scaffold Characteristics

- Structure: Open weave
- Maximum tensile strength: 800 N
- Average stiffness: 150N/mm

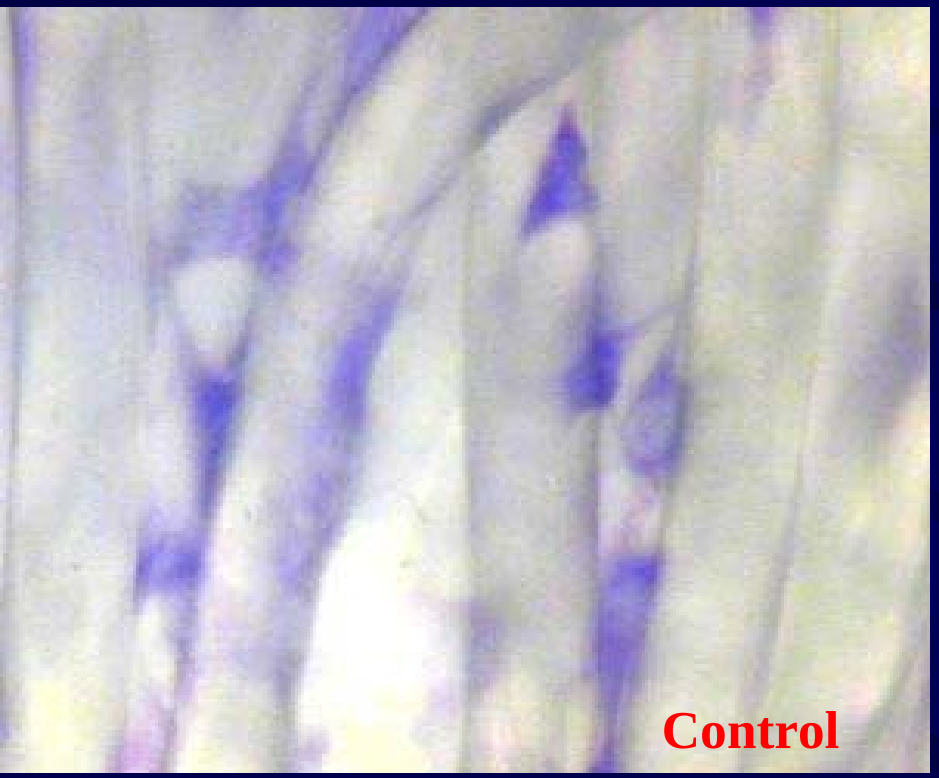


Apparatus



Raif el at. *Tissue Eng*. 13: 629-40. 2007

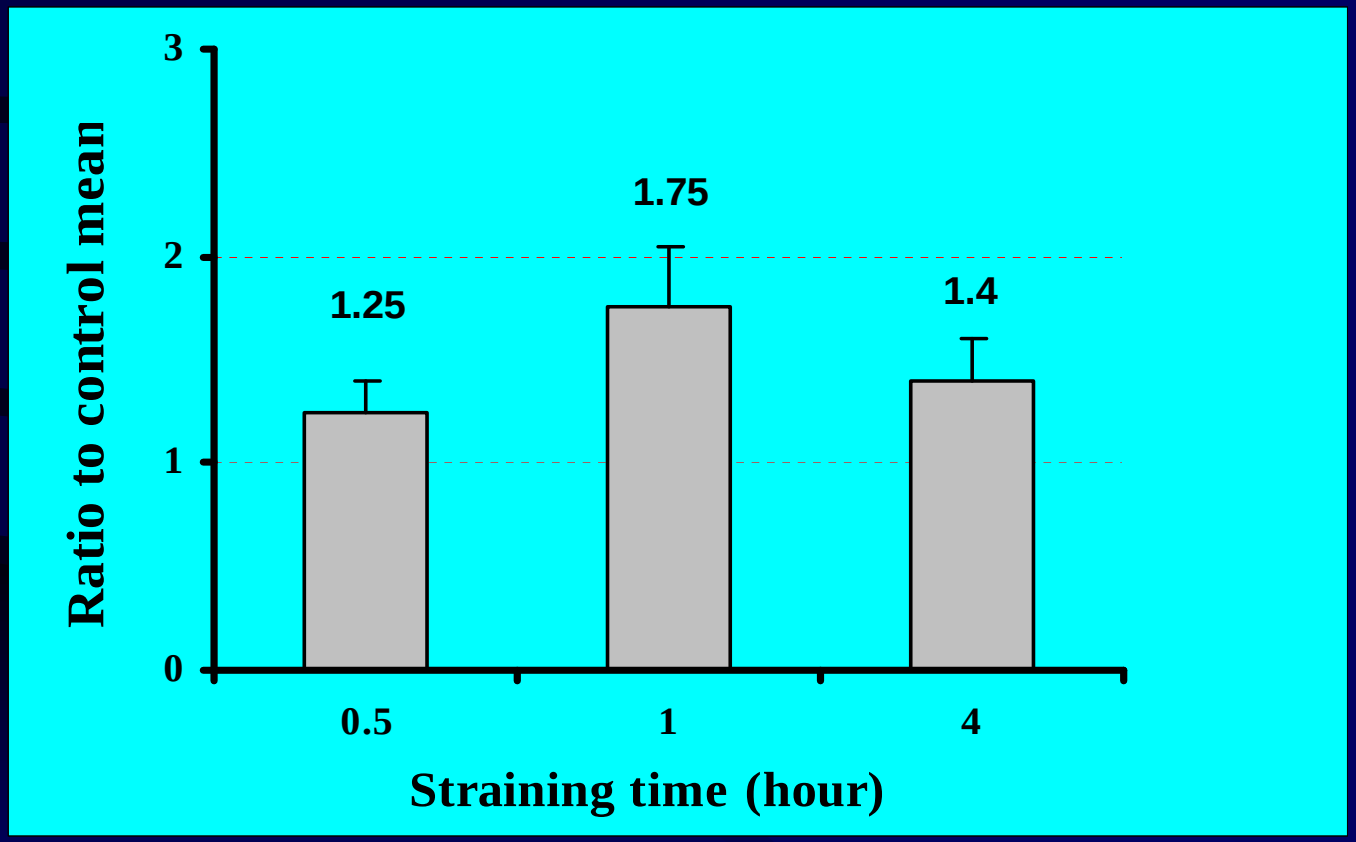
Effect of cyclic tensile strain on the cell shape



Short term effect of cyclic tensile load on cell proliferation

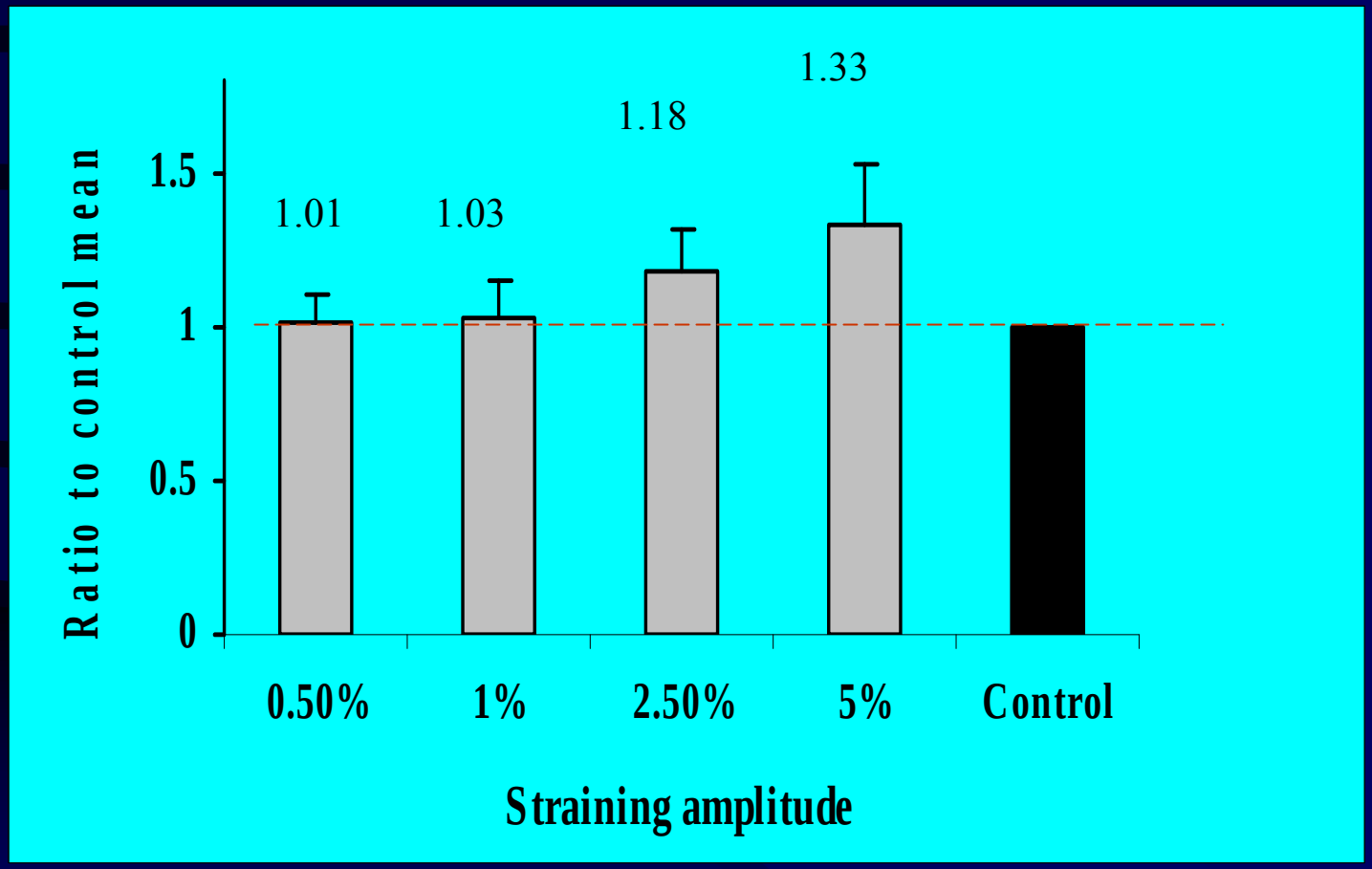
Effect of the strain duration on Cell proliferation

(Cyclic tensile strain of 5%)



Raif and Seedhom. ***Bone, 2005***

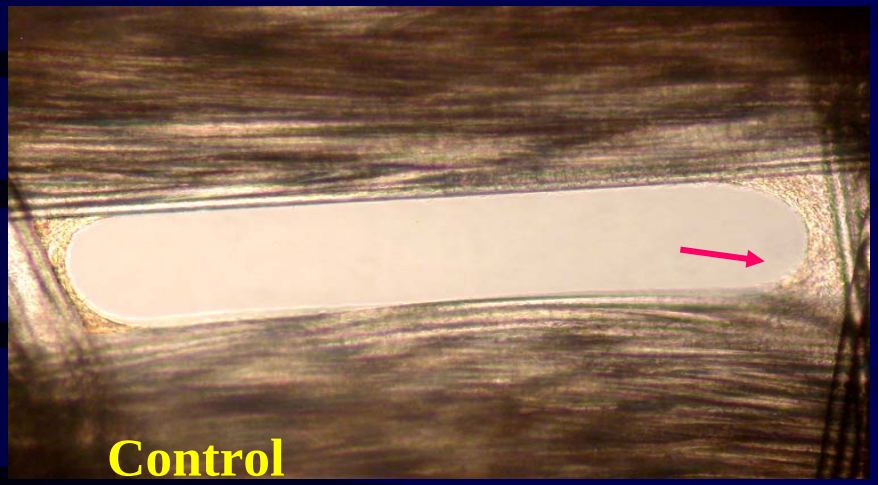
Effect of strain amplitude on cell proliferation

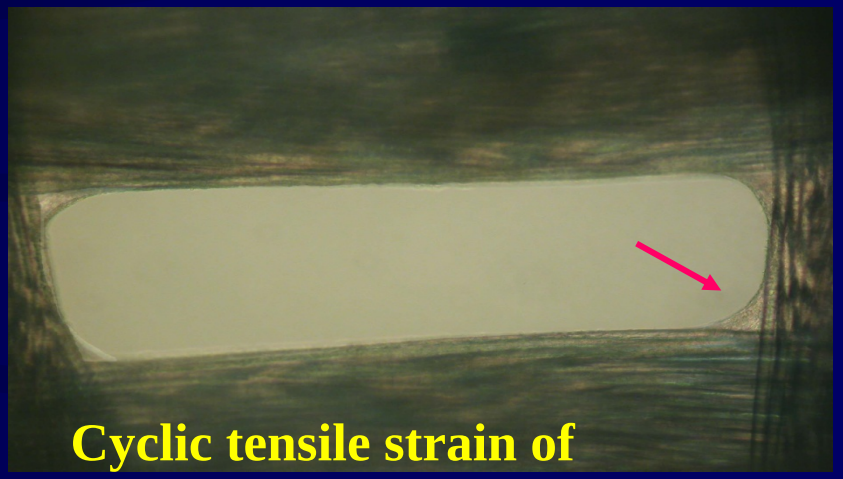


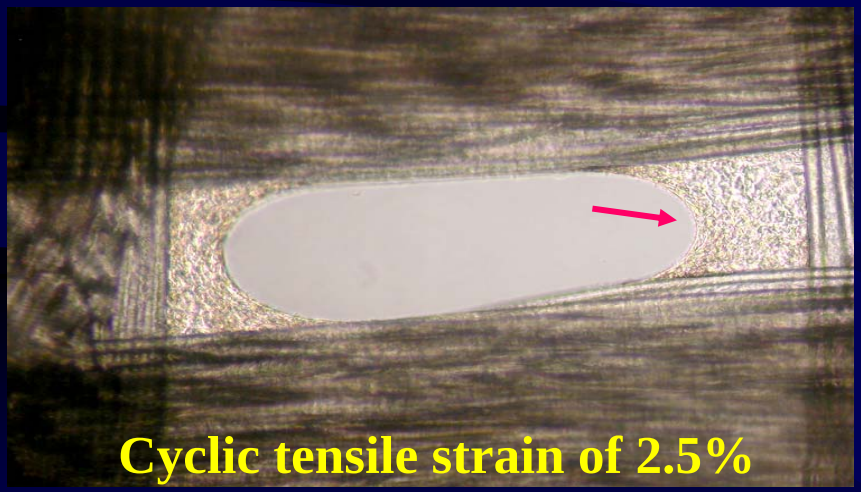
Raif and Seedhom . ***Bone , 2005***

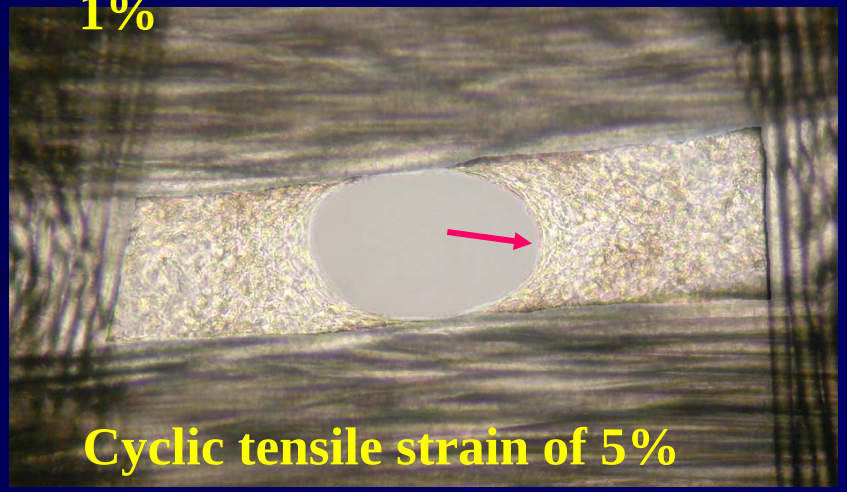
Medium term effect of cyclic tensile strain on synovial cells

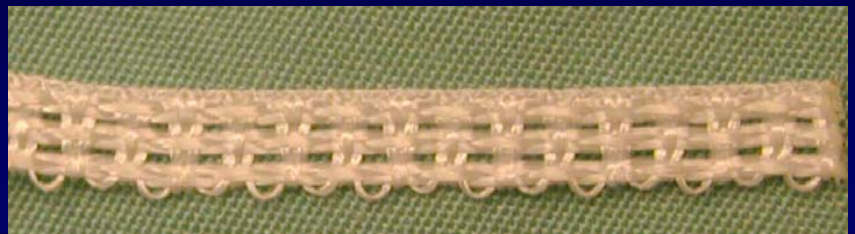
Tissue formation around PTPS





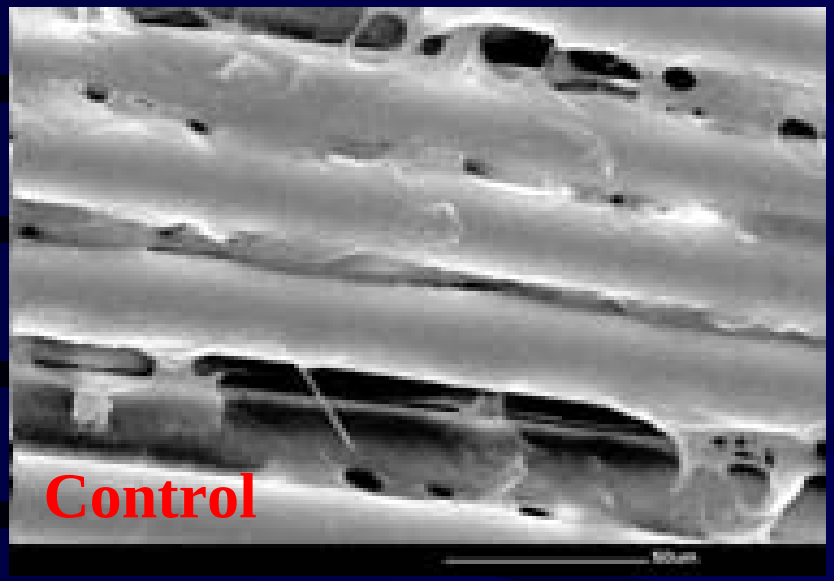


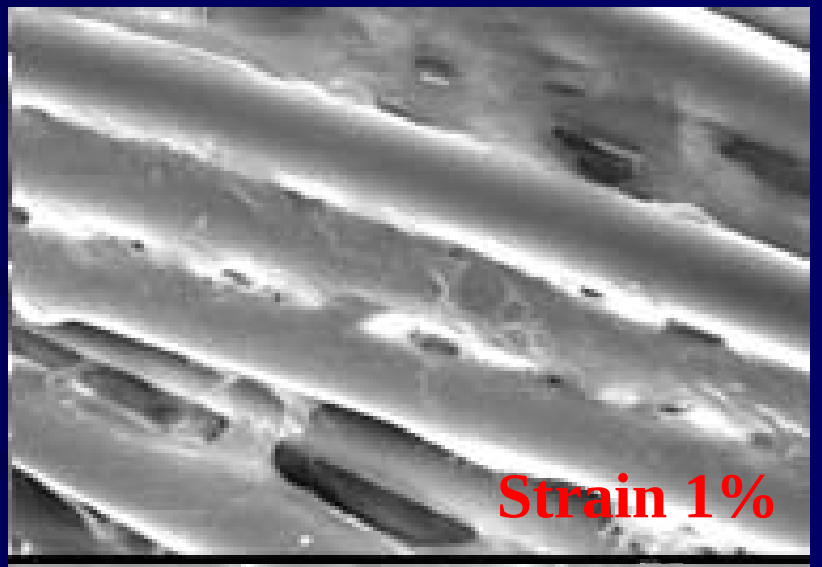


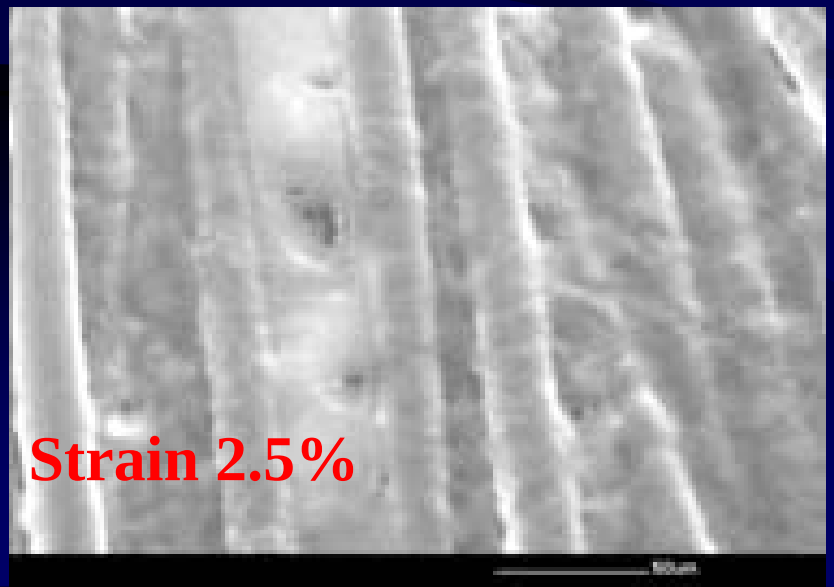


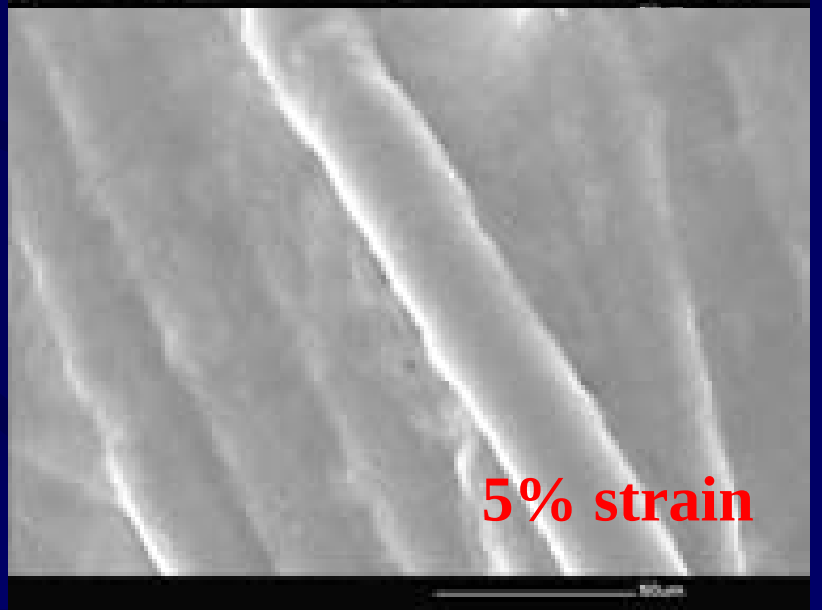
Raif and Seedhom. *Bone, 2005*

SEM micrographs of the synovial cells-seeded ligament scaffold after being subjected to a regime of cyclic tensile strain of 5% for 9 weeks

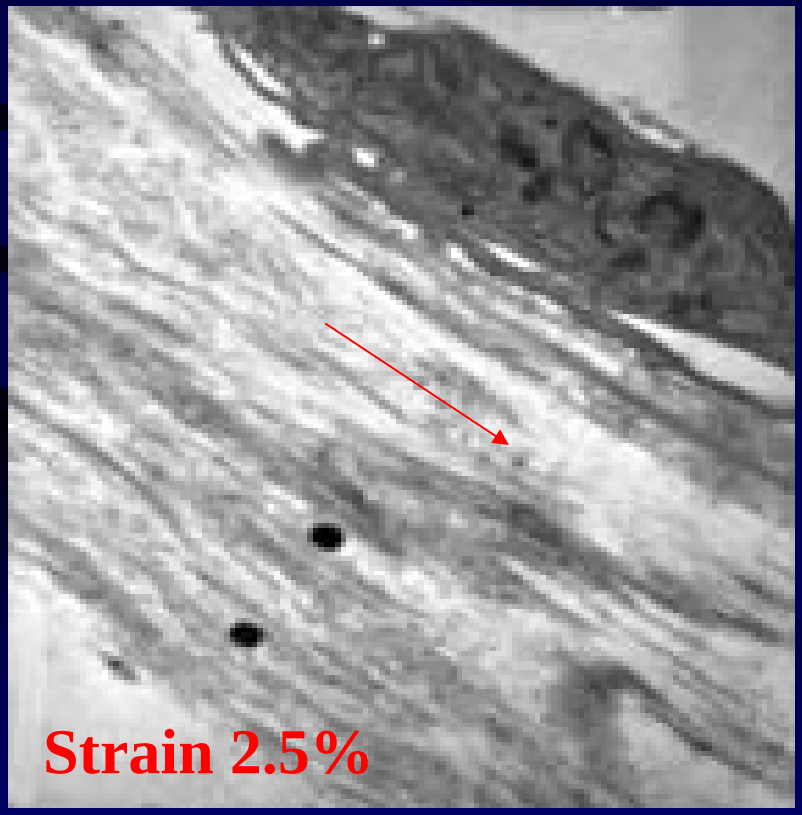


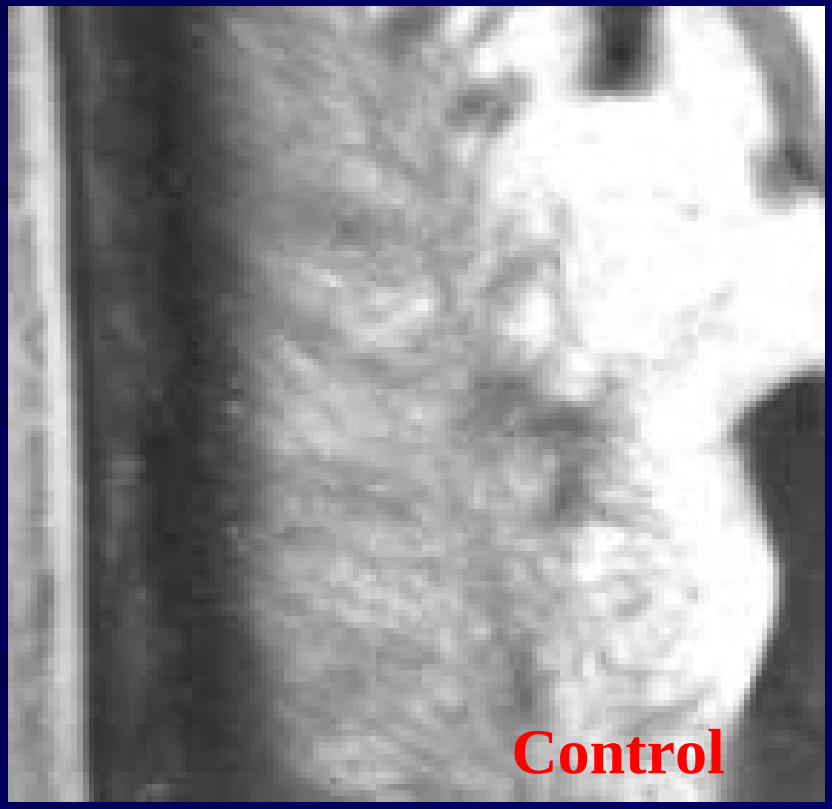






TEM showing fibres aligned along the direction of strain application on scaffolds seeded with synovial cells after being subjected to a 2.5% cyclic tensile strain for 9 weeks





Conclusion

- *Has cyclic tensile strain any effect on cell proliferation?*

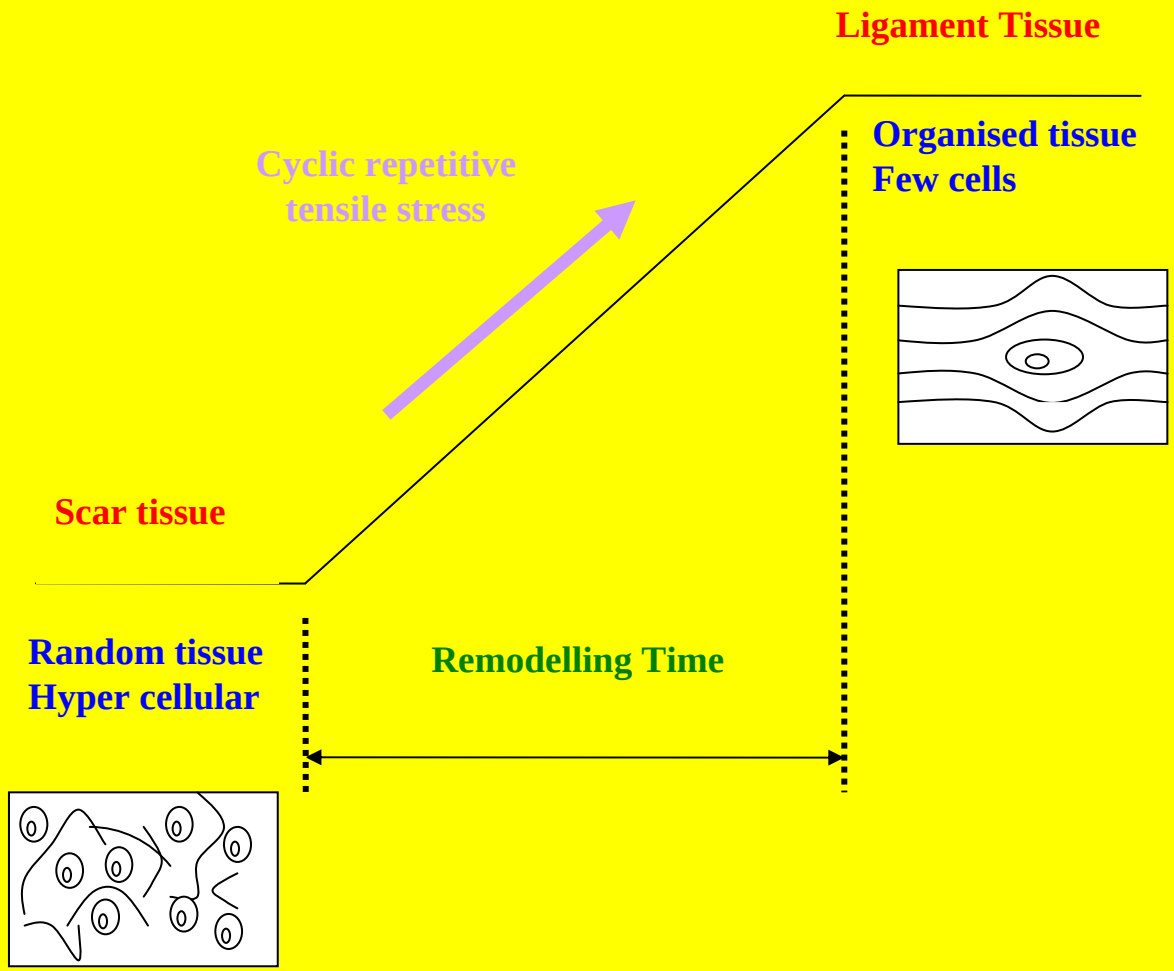
Yes

- Whether there is a threshold for *strain?*

Yes; Cyclic tensile strain of 1%

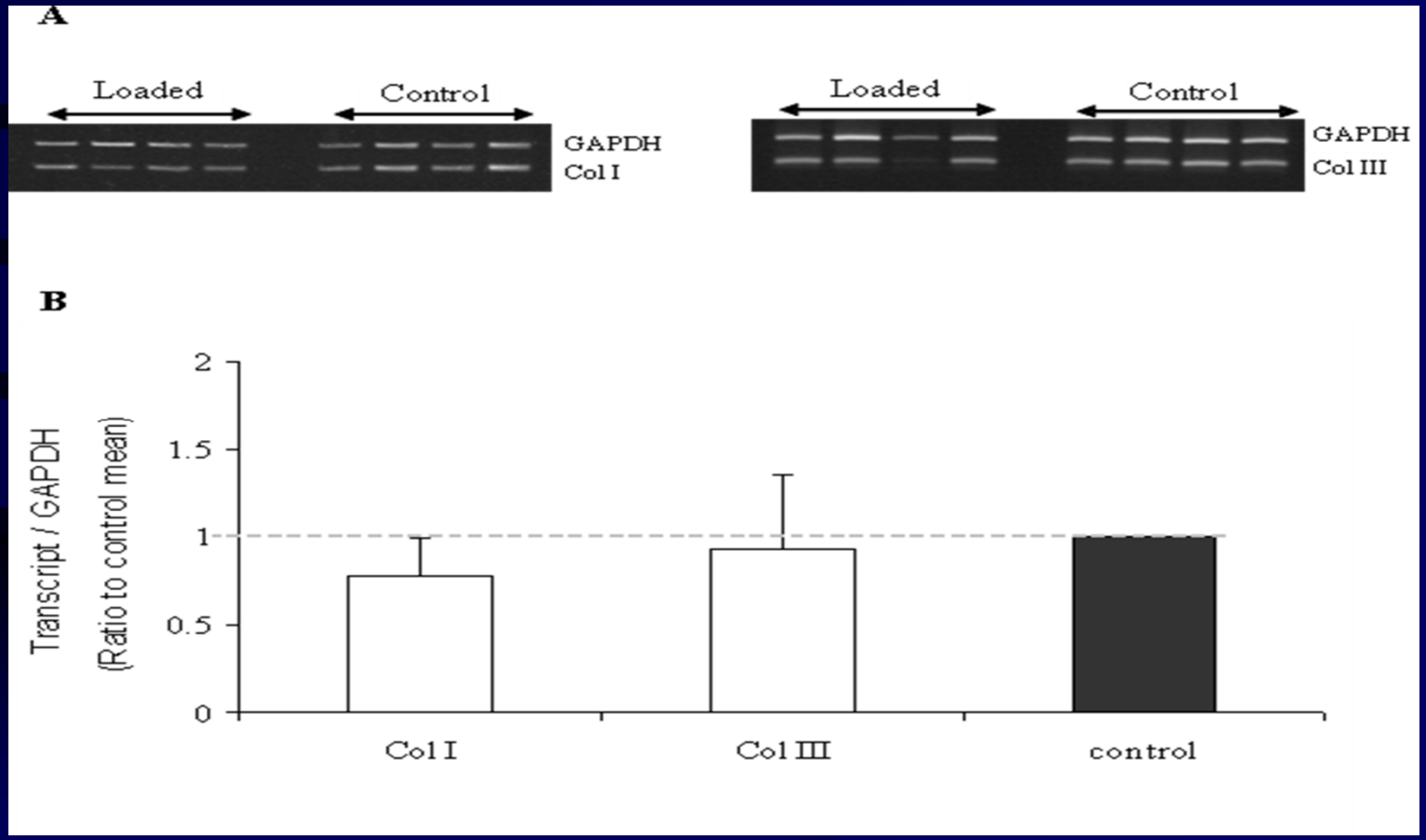
- Whether there is an effect of the periods of loading on the tissue induction process?

Yes; 1hour


Ligament Tissue
Organised tissue
Few cells
Cyclic repetitive
tensile stress
Scar tissue
Random tissue
Hyper cellular
Remodelling Time

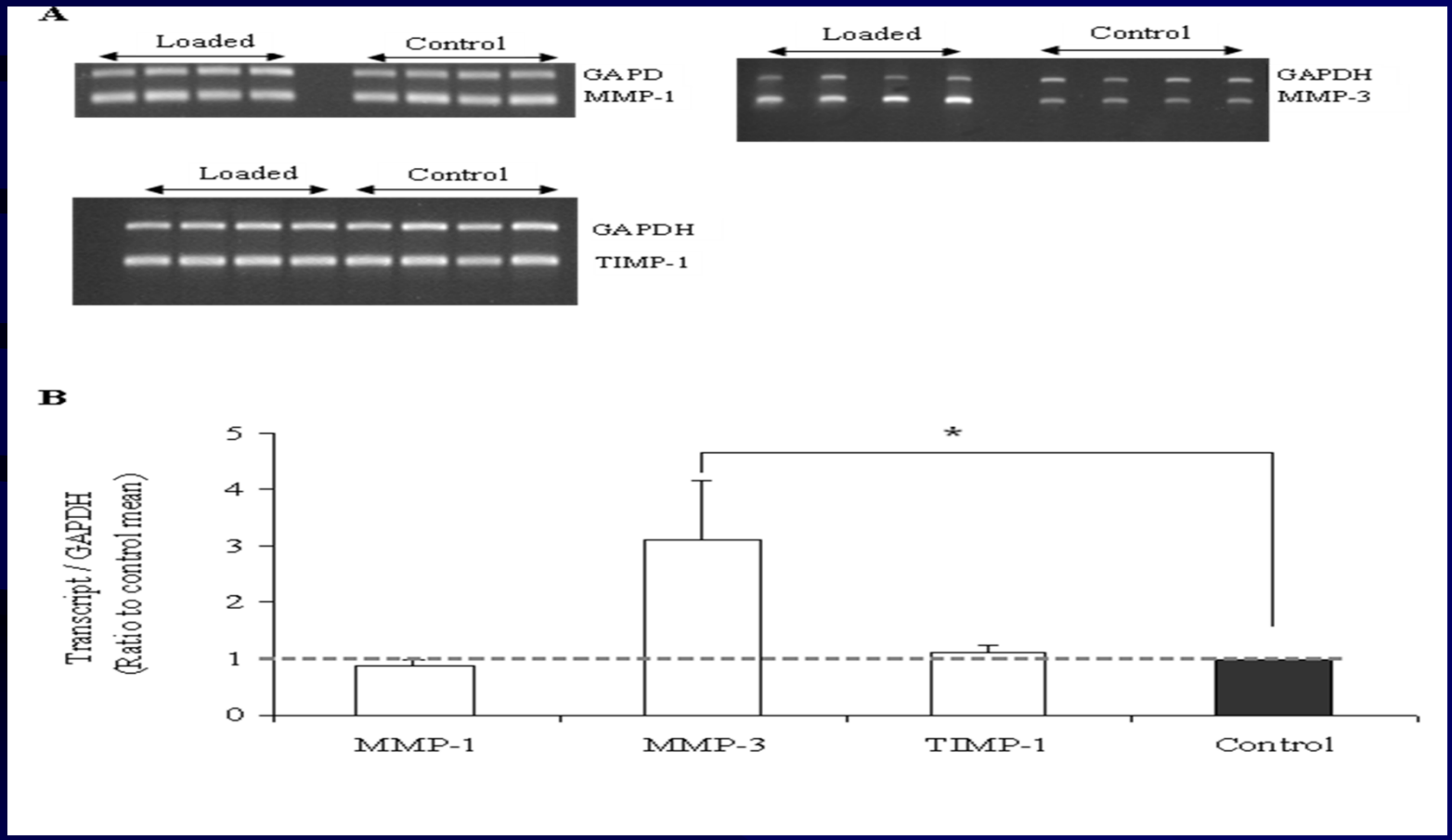
Short term effect of cyclic tensile load on the gene expression

Effect of cyclic tensile strain on type I and type III collagen expressions



Raif . *J Cell. Mol. Med., 2008*

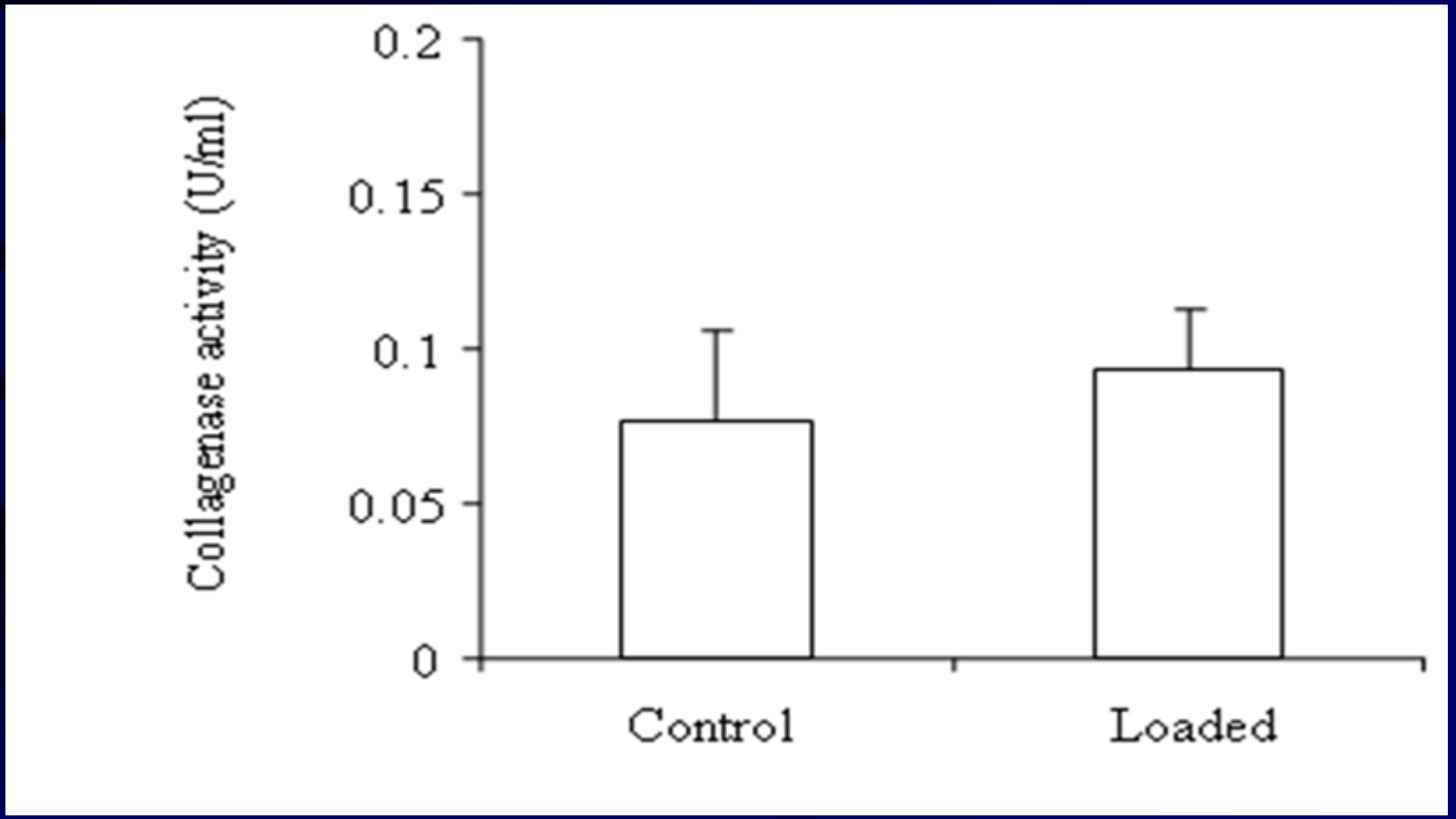
Effect of cyclic tensile strain on MMP-1 and MMP-3 expressions



Raif . ***J Cell. Mol. Med.,*** ***2008***

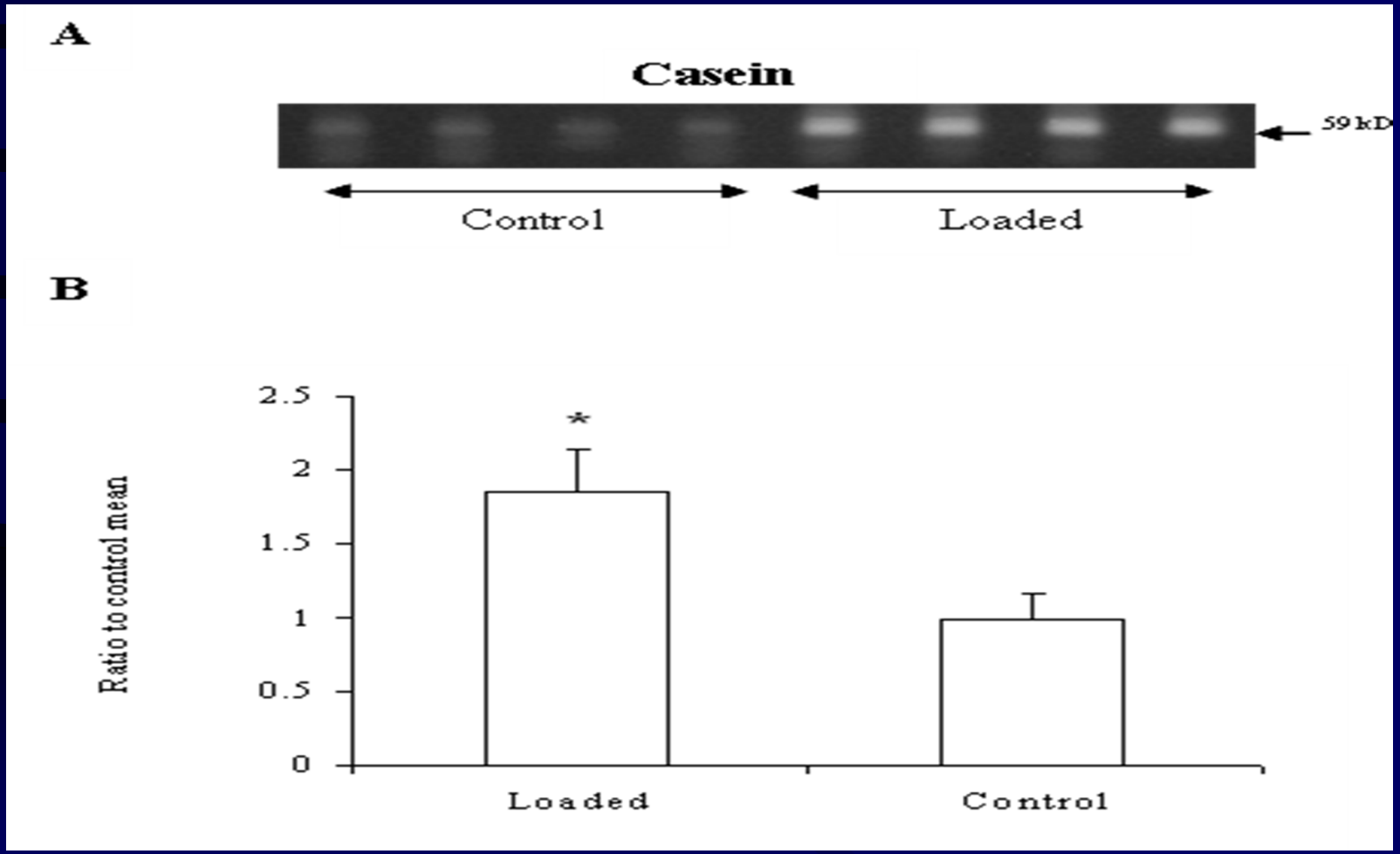
- In order to ascertain whether changes in mRNA level for gene such as MMP-3 would reflect changes in activity level, zymography was performed to verify the caseinolytic activity (MMP-3)

Effects of mechanical strain on collagenase activity of MMP-1

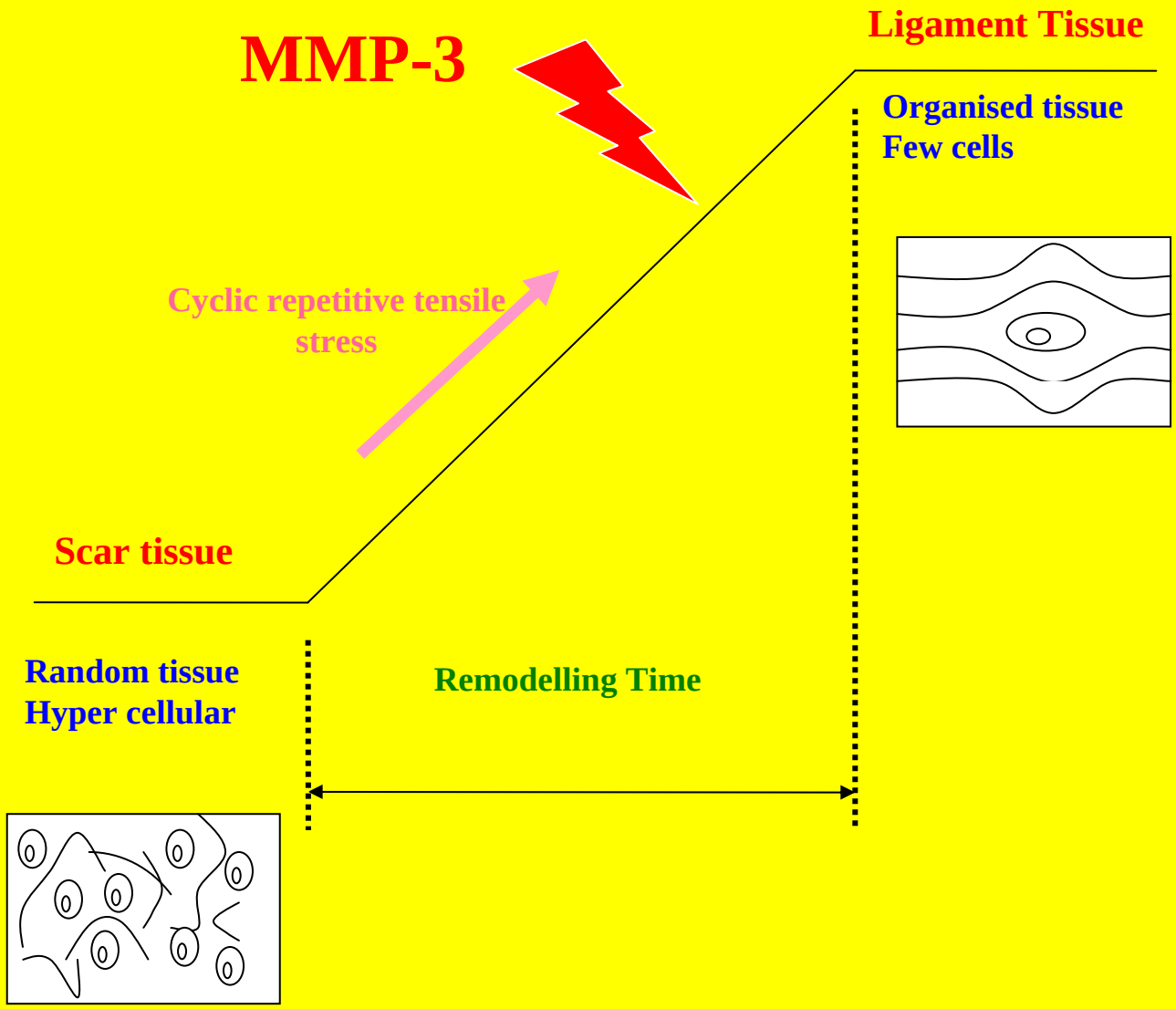


Raif . ***J Cell. Mol. Med., 2008***

Effects of mechanical strain on MMP-3 activity as determined by zymography



Raif . ***J Cell. Mol. Med,*** ***2008***


MMP-3
Ligament Tissue
Organised tissue
Few cells
Cyclic repetitive tensile stress
Scar tissue
Random tissue
Hyper cellular
Remodelling Time

Hypothesis

- MMP-3 may mediate the proteolysis of certain substrates surrounding the ligament scaffold. This will play critical role in facilitating cell migration, proliferation and tissue remodelling by breaking down the provisional tissue formed by the synovium, and by generating factors that induce chemotactic cell migration

Acknowledgment

- Dr Bahaa Seedhom — Academic Unit of Musculoskeletal and Rehabilitation, Bioengineering, University of Leeds, UK
- Mike Pullan — Academic Unit of Musculoskeletal and Rehabilitation, Bioengineering, University of Leeds, UK
- Jackie Hudson: — Leeds Dental Institute, Oral Biology, University of Leeds, UK

Sponsor: ARC

Thank you

شكرا

Merci

Gracias

感謝卡