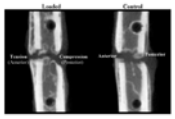
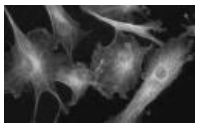
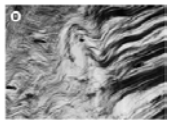
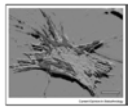




Soft Tissue Healing in Injuries Affecting the Knee Joint

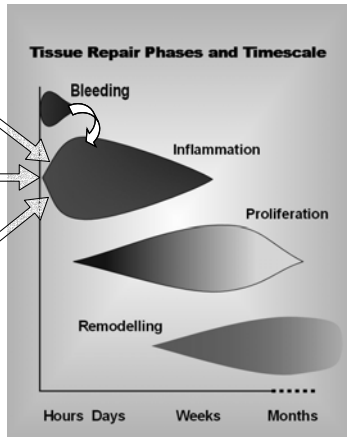
PhysioFirst – Kneed to Know – April 2007

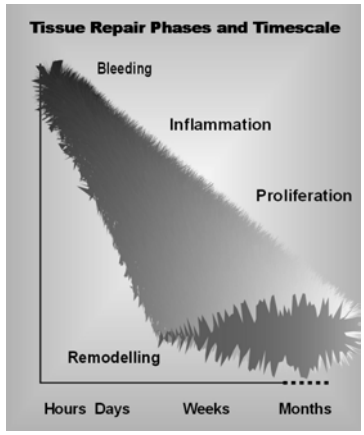
Professor Tim Watson
University of Hertfordshire
www.electrotherapy.org

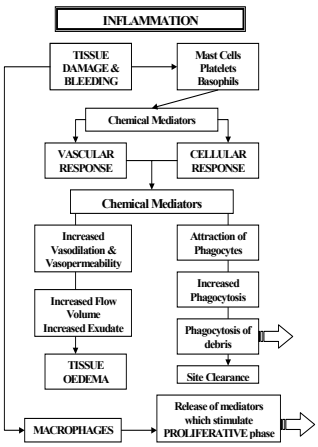


Topic Areas

- Review general phase model of tissue repair
- Identify the key issues in each of the inflammatory, proliferative and remodelling stages
- Consider the factors that influence repair
- Consider the potential impact of 'therapy' on these processes and events and possible additional biochemical / bioelectric influences







Phagocytes seeking and finding bacteria

Singer and Clark, 1999, NEJM, 341(10): 738-746

Cytokine	Major Source	Target Cells and Major Effects
Epidermal growth factor family		Epidermal and mesenchymal regeneration
Epidermal growth factor	Platelets	Pleiotropic-cell motility and proliferation
Transforming growth factor α	Macrophages, epidermal cells	Pleiotropic-cell motility and proliferation
Heparin-binding epidermal growth factor	Macrophages	Pleiotropic-cell motility and proliferation
Fibroblast growth factor family		Wound vascularization
Basic fibroblast growth factor	Macrophages, endothelial cells	Angiogenesis and fibroblast proliferation
Acidic fibroblast growth factor	Macrophages, endothelial cells	Angiogenesis and fibroblast proliferation
Keratinocyte growth factor	Fibroblasts	Epidermal-cell motility and proliferation
Transforming growth factor β family		Fibrosis and increased tensile strength
Transforming growth factors β1 and β2	Platelets, macrophages	Epidermal-cell motility, chemotaxis of macrophages and fibroblasts, extracellular-matrix synthesis and remodeling
Transforming growth factor β3	Macrophages	Antiscarring effects
Other		
Platelet-derived growth factor	Platelets, macrophages, epidermal cells	Fibroblast proliferation and chemoattraction, macrophage chemoattraction and activation
Vascular endothelial growth factor	Epidermal cells, macrophages	Angiogenesis and increased vascular permeability
Tumor necrosis factor α	Neutrophils	Pleiotropic expression of growth factors
Interleukin-1	Neutrophils	Pleiotropic expression of growth factors
Insulin-like growth factor 1	Fibroblasts, epidermal cells	Reepithelialization and granulation-tissue formation
Colony-stimulating factor 1	Multiple cells	Macrophage activation and granulation-tissue formation

The Roles of Growth Factors in Tendon and Ligament Healing

Tendon healing is a complex and highly-regulated process that is initiated, sustained and eventually terminated by a large number and variety of molecules. Growth factors represent one of the most important of the molecular families involved in healing, and a considerable number of studies have been undertaken in an effort to elucidate their many functions.

Five growth factors are reviewed whose activities have been best characterised during tendon healing: insulin-like growth factor-I (IGF-I), transforming growth factor β (TGFβ), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF).

Molloy et al (2003) Sports Med 33(5);381-394

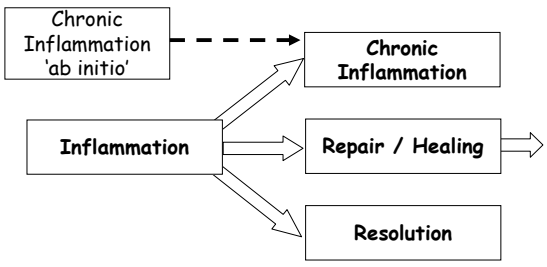
Cellular Responses to Electrical Stimulation

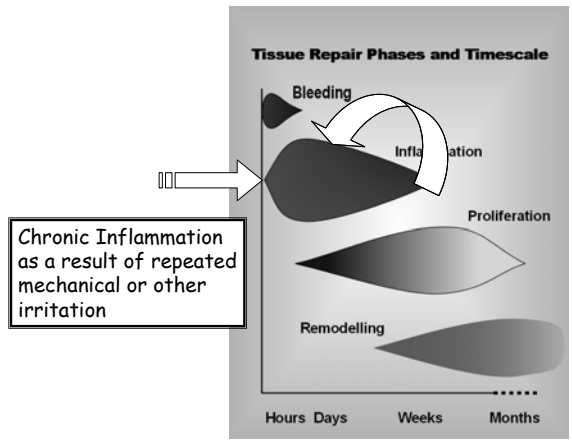
Cell Type	References
Fibroblasts	Bourguinon and Bourguinon, 1987; Goldman and Pollack, 1996; Canseven and Atalay, 1996; Thawer and Houghton, 2001; Reger et al, 1999; Cheng and Goldman, 1998
Neutrophils	Fukushima et al, 1953; Kloth, 1996; Gentzkow and Miller, 1991
Macrophages	Orida and Feldman, 1982; Cho et al, 2000
Mast Cells	Reich et al, 1991; Gentzkow, 1993; Taskan et al, 1997
Endothelial Cells	Nissen et al, 1998; Goldman et al, 2004; Zhao et al, 2004; Li et al, 2002; Chang et al, 1996
Myofibroblasts	Gabbiani et al, 2003; Brown and Gogia, 1987
Epidermal Cells	Nishimura et al, 1996; Sheridan et al, 1996; Zhao et al, 1999; Nuccitelli, 2003; Pullar et al, 2001; Farbould et al, 2000

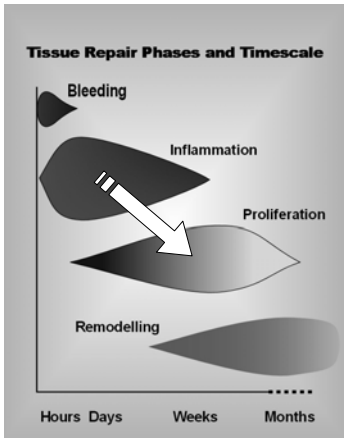
Examples of Mediators Influenced by Therapy

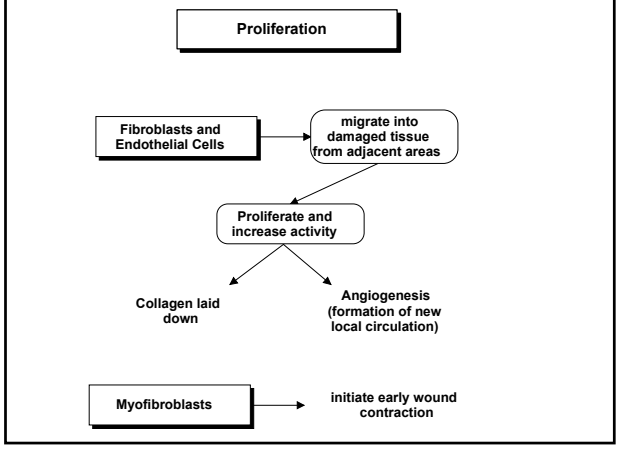
- **Ostrowski et al (2000) – link between exercise and Interleukin-6 (IL-6) production**
- **Leung et al (2006) – link between ultrasound and TGF-β in knee ligament healing**
- **Zhang et al (2004) – demonstrated link between electroacupuncture and peripheral inflammatory responses**
- **Bjordal et al (2006) – link between laser therapy and altered prostaglandin levels in the tissue (Achilles tendon)**

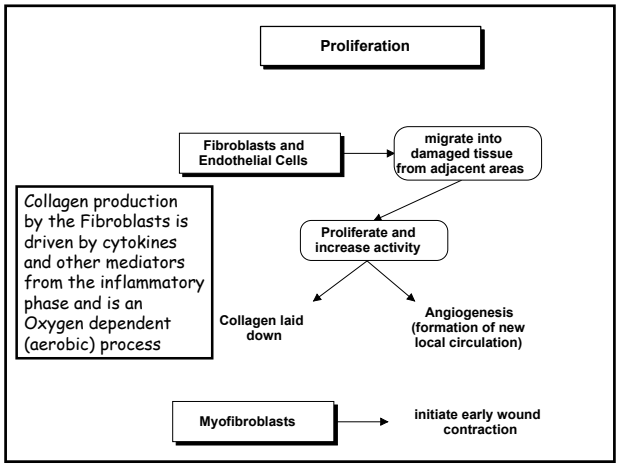
Progress of Inflammation

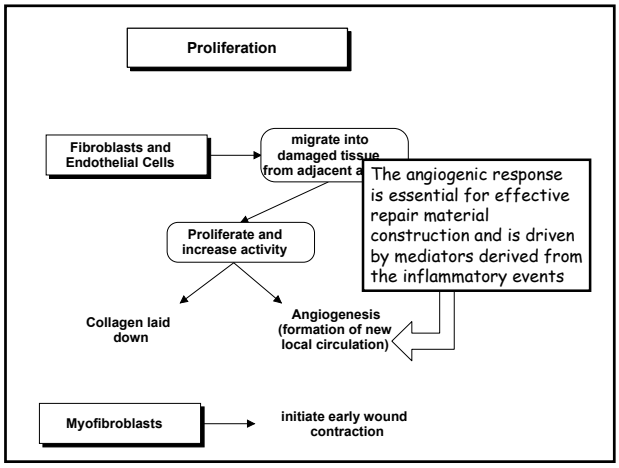












Review

The vasculature and its role in the damaged and healing tendon

Steven A Fenwick[1], Brian L Hazleman[2] and Graham P Riley[2]

Fenwick et al 2002. Arthritis Research 4(4); 252-260

. . . Tendon is a comparatively poorly vascularised tissue that relies heavily upon synovial fluid diffusion to provide nutrition. . .

. . . During tendon injury, as with damage to any tissue, there is a requirement for cell infiltration from the blood system to provide the necessary reparative factors for tissue healing . . . .

Fenwick et al (2002) contd

- **Review the response of the vasculature to tendon damage in a number of forms, and how and when the revascularisation or neovascularisation process occurs.**
- **Also review on the revascularisation of tendon during its use as a tendon graft in both ligament reconstruction and tendon–tendon grafting . . . . .**

ELSEVIER

Journal of Orthopaedic Research 20 (2002) 984–989

Journal of Orthopaedic Research

www.elsevier.com/locate/orthres

Vascular physiology and long-term healing of partial ligament tears

Robert C. Bray *, Catherine A. Leonard, Paul T. Salo

Looked at BLOOD FLOW and LIGAMENT RESPONSE to injury (rabbit model)

Various models for ACL and MCL trauma including control, sham operated, partial MCL, partial ACL and complete PCL transection giving rise to knee joint instability

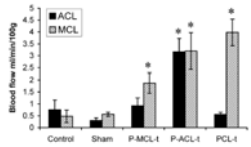
In control animals, the baseline blood flow for the MCL was not significantly different from that of the ACL

Sham operation did not induce any significant change in ligament blood flow at 16 weeks

Both MCL and ACL showed significant increases in blood flow 16 weeks after hemisection (389% and 421% of control, respectively).

Both ligaments therefore demonstrate the ability to adapt to the requirements of tissue remodelling with changes in vascular physiology.

Fig. 1. Standardized blood flow in rabbit MCL and ACL in control animals and 16 weeks after sham operation, partial MCL injury, partial ACL injury or complete PCL transection. Values are given as mean ± SEM ($n = 6$ per group). Asterisks indicate significant difference from control or sham, comparing each ligament to its own control values (ANOVA, $p < 0.05$).



Inflammatory Response of Human Tendon Fibroblasts to Cyclic Mechanical Stretching

Zhaozhu Li, MD, Guoguang Yang, MS, Mustafa Khan, MD, David Stone, MD, Savio L-Y. Woo, PhD, and James H-C. Wang,* PhD

Zhaozhu et al 2004 : Am J Sports Med 32(2);435-440

- **Looked at effect of fibroblast stretching on 2 mediators – leukotriene B4 and prostaglandin E2**
- **Both related to inflammatory events and tissue repair**
- **Human patellar tendon fibroblasts**
- **Also considered influence of NSAID**

Zhaozhu et al 2004 (contd)

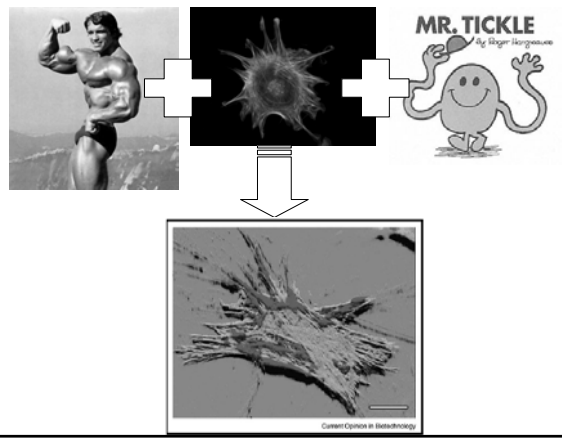
. . . CONCLUSION . . .

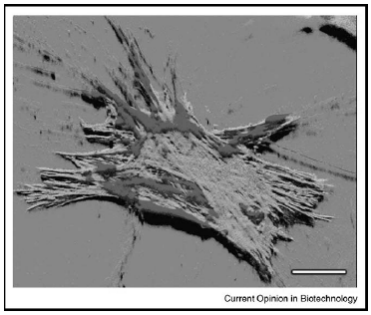
Cyclic stretching of human tendon fibroblasts increases the production of prostaglandin E2 and leukotriene B4 which enhance repair process

Also demonstrated that NSAID has adverse effect and that its use might contribute to the development of tendinopathy

Angiogenic Influence of existing Therapies

- **Azuma et al (2001) demonstrate that LIPUS influences angiogenesis in relation to # healing**
- **Reher et al (2002) demonstrate influence of ultrasound in relation to NO and PGE2 production**
- **Zhao et al (2004) demonstrate link between electrical stimulation and angiogenic enhancement by means of VEGF mediated response**





From Hinz & Gabbiani (2003)
Mechanisms of force generation and transmission by myofibroblasts
Current Opinion in Biotechnology 14;538-546

Gabbiani, G. (2003). "The myofibroblast in wound healing and fibrocontractive diseases." J Pathol 200(4): 500-3

- Fibroblastic cells acquire contractile features during healing, modulating into myofibroblasts
- Myofibroblasts synthesize extracellular matrix components such as collagen types I and III
- The transition from fibroblasts to myofibroblasts is influenced by *mechanical stress*, TGF-beta and cellular fibronectin.

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Journal of Orthopaedic Research 20 (2002) 953–956
Journal of Orthopaedic Research
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Fibroblast orientation to stretch begins within three hours

C. Neidlinger-Wilke [a], E. Grood [b], L. Claes [a], R. Brand [c,*]

Connective tissue cells align in response to stretch. Studies have shown these responses occur within 12-14 h of initiation of stretch, but do not identify the time at which this orientation occurs
Nor whether the orientation continues after cessation of stretch.
Study to establish the earliest times at which fibroblast orientation occurs, using cultured primary human fibroblasts on deformable culture dishes and stretched them for up to 24 h. (1 Hz, 8% uniaxial strain)

Neidlinger-Wilke et al 2002 (contd)

Cells photographed for up to 24 h after cessation of stretch

The fibroblasts began to orient by 2-3 h and orientation appeared nearly complete by 24 h.

Cultures stretched for 2 or 3 h continued to exhibit greater degrees of orientation (compared to controls) for 2 or 3 h respectively after cessation of stretch.

Conclude that fibroblasts begin to orient within 3 h of initiation of stretch, and that they continue to orient for several hours after cessation of stretch.

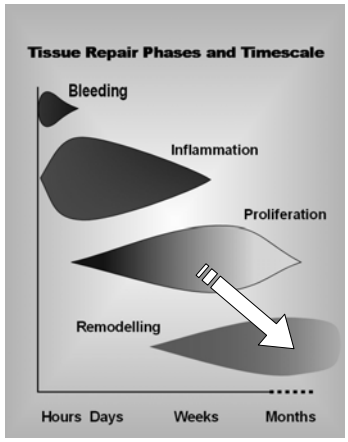
ELSEVIER
Journal of Orthopaedic Research 22 (2004) 847–853
Journal of Orthopaedic Research
www.elsevier.com/locate/orthres

Cyclic strain influences the expression of the vascular endothelial growth factor (VEGF) and the hypoxia inducible factor 1 alpha (HIF-1α) in tendon fibroblasts ☆

Wolf Petersen [a,*], Deike Varoga [b], Thore Zantop [b], Joachim Hassenpflug [b], Rolf Mentlein [c], Thomas Pufe [c]

Neovascularization is involved in beneficial and detrimental processes of tendon pathology. We investigated the influence of repetitive motion on the expression of the most important angiogenic factor, the vascular endothelial growth factor (VEGF)

These results demonstrate that mechanical factors are involved in the regulation of VEGF expression in tendon tissue.



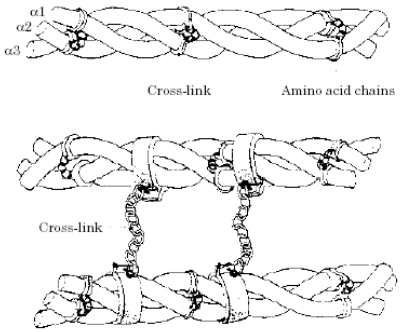


Fig. 3 Intramolecular (top) and intermolecular (bottom) collagen cross links. Reproduced by kind permission of Collamore Press from Curwin and Stanish.[1]

Collagen Bonding

- X Links (bonds)
  - within triple helix structure
  - between collagen filaments
- MORE BONDS :
  - more strength
  - more stiffness
  - less mobility
  - less extensibility

IMMOBILISATION & IMMOBILITY

- Increases number of bonds
- Decreases lubrication
- therefore :
  - decreased mobility
  - decreased flexibility
  - decreased extensibility
- Akerson et al 1977

Evans & Stanish (2000)
The basic science of tendon injuries
Current Orthopaedics 14;403-412

Immobilization

Immobilization atrophies tendon. The deleterious changes include:

- reduced synthesis of new collagen;
- thinner, disorientated collagen fibres;
- reduced number and size of cross links;
- reduced proteoglycan content;
- tenocyte degeneration.

The tensile strength, elastic stiffness and total weight of tendon decrease. These alterations can be seen after a few weeks of disuse, occurring more rapidly than the adaptations induced by exercise. The effects are slower and less dramatic than those observed in muscle tissue, owing to the lower metabolic rate of tendon.[2]

Mechanobiology and diseases of mechanotransduction

Donald E Ingber

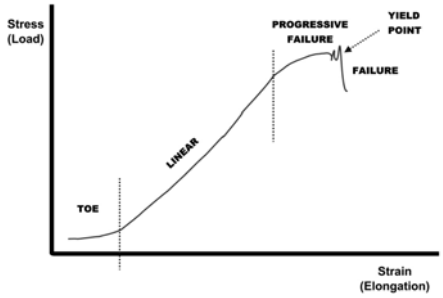
Ingber (2003) Ann Med 35;564-577

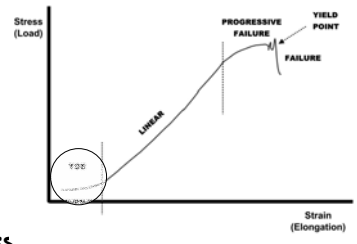
This article reviews the key roles that physical forces, extracellular matrix and cell structure play in the control of normal development, as well as in the maintenance of tissue form and function.
Recent insights into cellular mechanotransduction - the molecular mechanism by which cells sense and respond to mechanical stress - also are described.

Collagen Biomechanics

- Collagen fibre orientation such as to resist tension throughout its length
- non linear stress - strain relationship
- during normal physiological activity tendon / ligament is subjected to <25% of its ultimate stress

Collagen Stress / Strain Curve





TOE

- **early stress**
- **increase in length with minimal force applied**
- **relate to CRIMPS and possible FILAMENT SLIDING**

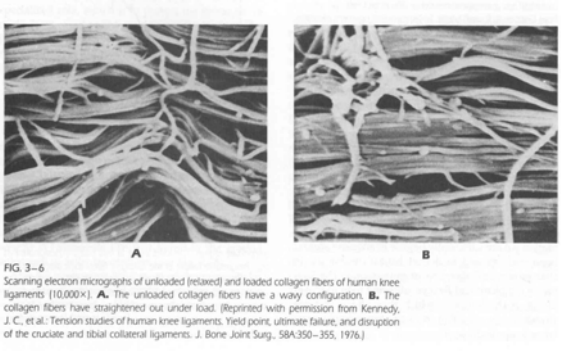
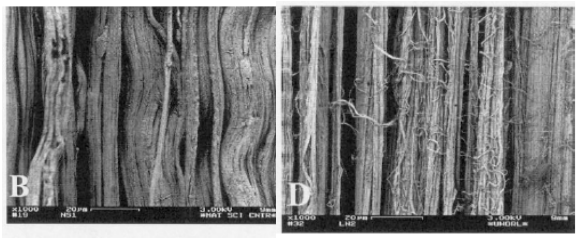


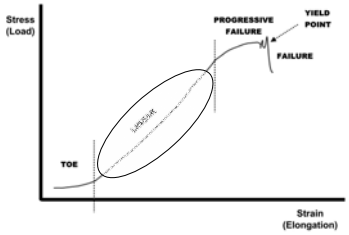
FIG. 3–6
Scanning electron micrographs of unloaded (relaxed) and loaded collagen fibers of human knee ligaments (10,000×). **A.** The unloaded collagen fibers have a wavy configuration. **B.** The collagen fibers have straightened out under load. (Reprinted with permission from Kennedy, J. C., et al.: Tension studies of human knee ligaments. Yield point, ultimate failure, and disruption of the cruciate and tibial collateral ligaments. J. Bone Joint Surg., 58A:350–355, 1976.)

from Kennedy et al (1976) JBJS 58(A); 350-355



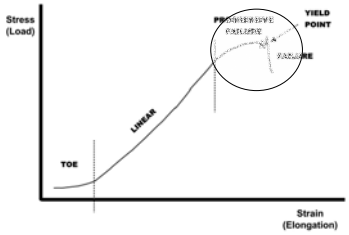
Unloaded ligament **(x1000)** **Loaded ligament**

Hurschler et al (2003)
Connective Tissue Research 44;59-68



LINEAR

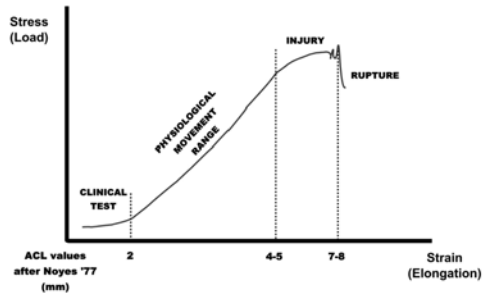
- **almost linear increase in resistance as the load is increased**



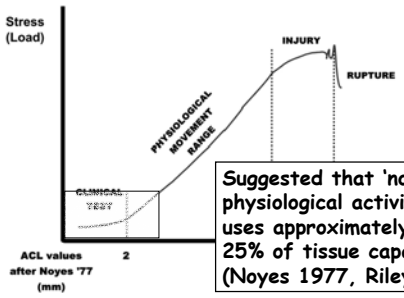
FAILURE

- **progressive failure beyond a certain point**
- **fibres begin to fail**
- **eventual and rapid onset of complete failure at yield point**

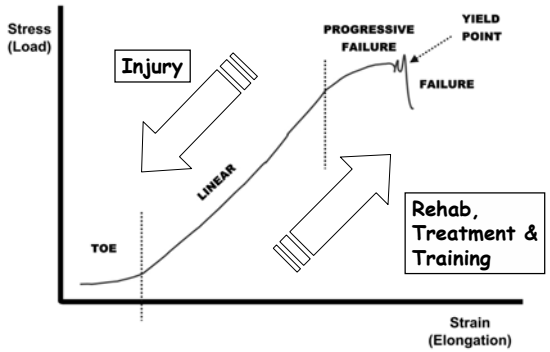
Clinical Correlates (after Noyes 1977)



Clinical Correlates



Suggested that 'normal' physiological activity only uses approximately 25% of tissue capacity (Noyes 1977, Riley 2004)



Stress / No Stress

- Bone - tendon - ligament ADAPT to the demands put on them
- Increased stress - become stronger and stiffer
- Decreased stress - become weaker and less stiff

Effect of Exercise

- Evidence that the use of exercise can increase tendon tensile stress
- Tipton et al 1967, 1970
- Viidik 1969, 1979 Woo 1980, 1982
- Evidence that the use of exercise can increase ligament / bone junction strength
- Tipton et al 1967, Viidik 1968, Cabaud et al 1980, Woo et al 1981

Evans & Stanish (2000)
The basic science of tendon injuries
Current Orthopaedics 14;403-412

Exercise

Exercise-induced structural and metabolic adaptations include:

- increased tenoblast activity;
- accelerated collagen synthesis;
- increased collagen fibre thickness, cross links and crimp angle;
- improved stress orientation of fibres;
- larger diameter and total weight.

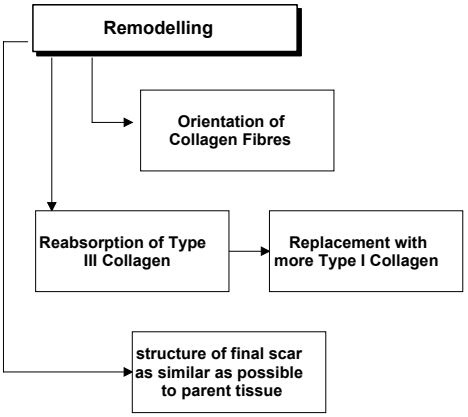
These changes improve mechanical properties, producing a tendon with increased stiffness and ultimate strength, capable of absorbing a larger amount of energy before failure. The tendon becomes larger, stronger, and more resistant to injury.

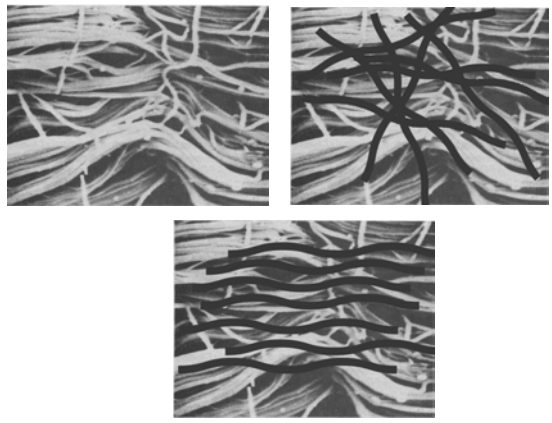
Effect of Exercise

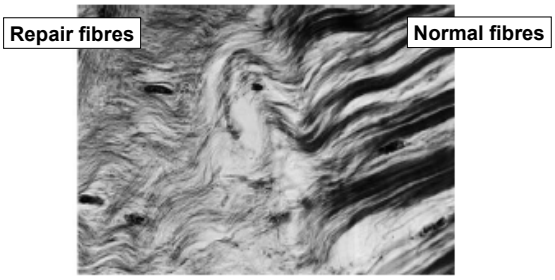
- Evidence that the collagen bundle diameter increases with stress / exercise
- Immobilisation / immobility reduces the tensile strength of ligament / tendon

Noyes (1977)

- Monkey ACL research
- Had previously demonstrated 40% reduction in ACL strength following 6/52 immobilisation
- 8/52 immobilisation in POP
- POP off – followed by reconditioning programme (exercise)
- 5/12 later , ligaments still 20% reduced strength
- 12/12 – 'almost' same as the controls (90%)



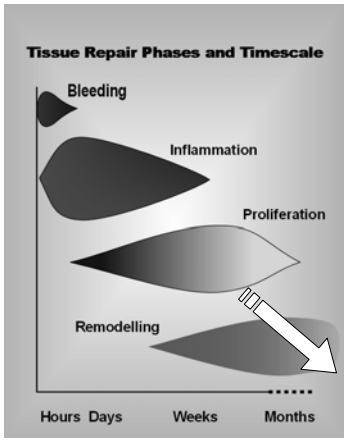


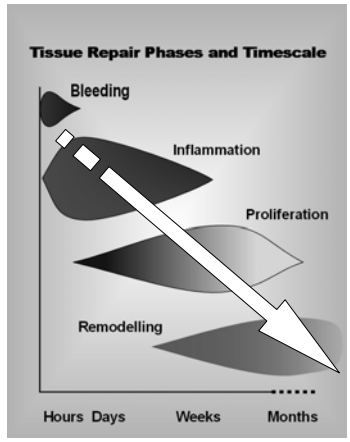


Light micrograph illustrating ruptured collagen fibres with no regular crimp pattern 1cm from the macroscopical rupture site in the ruptured Achilles tendon (mag x400) Jarvinen et al (2004) J Orthop Res 22;1303-1309

Influences of Therapy on Remodelling

- Neidlinger Wilke et al (2002) and Ingber (2003) – mechanical influence
- Also link with fibroblast responses to mechanical stress
- Other therapies – e.g. Ultrasound, laser therapy and electrical stimulation have been shown to influence these events (e.g. Nussbaum, 1998, Huys et al, 1992 and Byl et al, 1996)





Implications for Therapy

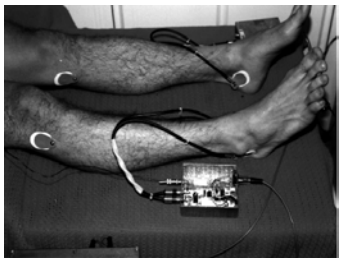
- Mechanical stress influences not only the mechanical but also the biochemical environment of repairing tissue
- Therefore exercise and manual therapy have the POTENTIAL to influence repair at a level not usually described
- Of course, more research needed in order to 'fill the gap'

Bioelectric Potential

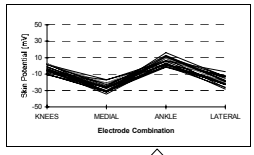
- Bioelectric influences *could* act in addition to the mechanical, neurological and biochemical influences of manual and exercise therapy
- Established relationship(s) between ENDOGENOUS BIOELECTRIC POTENTIALS and tissue injury/repair (Watson 1995, 2000, 2002, 2006)
- Relationships between therapy intervention and bioelectrics need further investigation

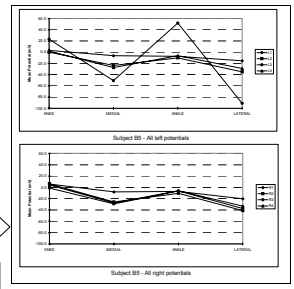
Bioelectricity

- Human natural bioelectric phenomena
- Relations between tissues injury, healing and bioelectric events
- Voluntary control over bioelectric events



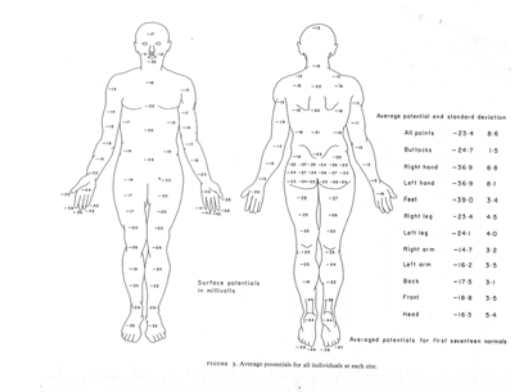
Lower Limb Bioelectricity after Injury





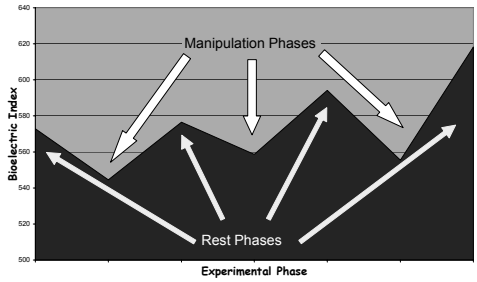
Normal Bioelectric Pattern

Injured Bioelectric Pattern



Skin Potential maps after Foulds and Barker 1983

Bioelectric Influences with Manual Therapy



Summary and Conclusion

Tissue repair

- The process of repair is a highly regulated and complex cascade of events
- The division into 'phases' is convenient rather than 'real'
- The increased understanding of the effect of mediators in this scenario offers an opportunity to further influence the events and enhance the processes

Repair and Therapy

- In addition to the 'known' effects of exercise, electrotherapy and manual therapy, new possibilities are arising with current findings
- Existing therapies have effects that have not been previously determined
- Opportunity to exploit these further and to have even greater potential to enhance the process of repair

Thank You

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