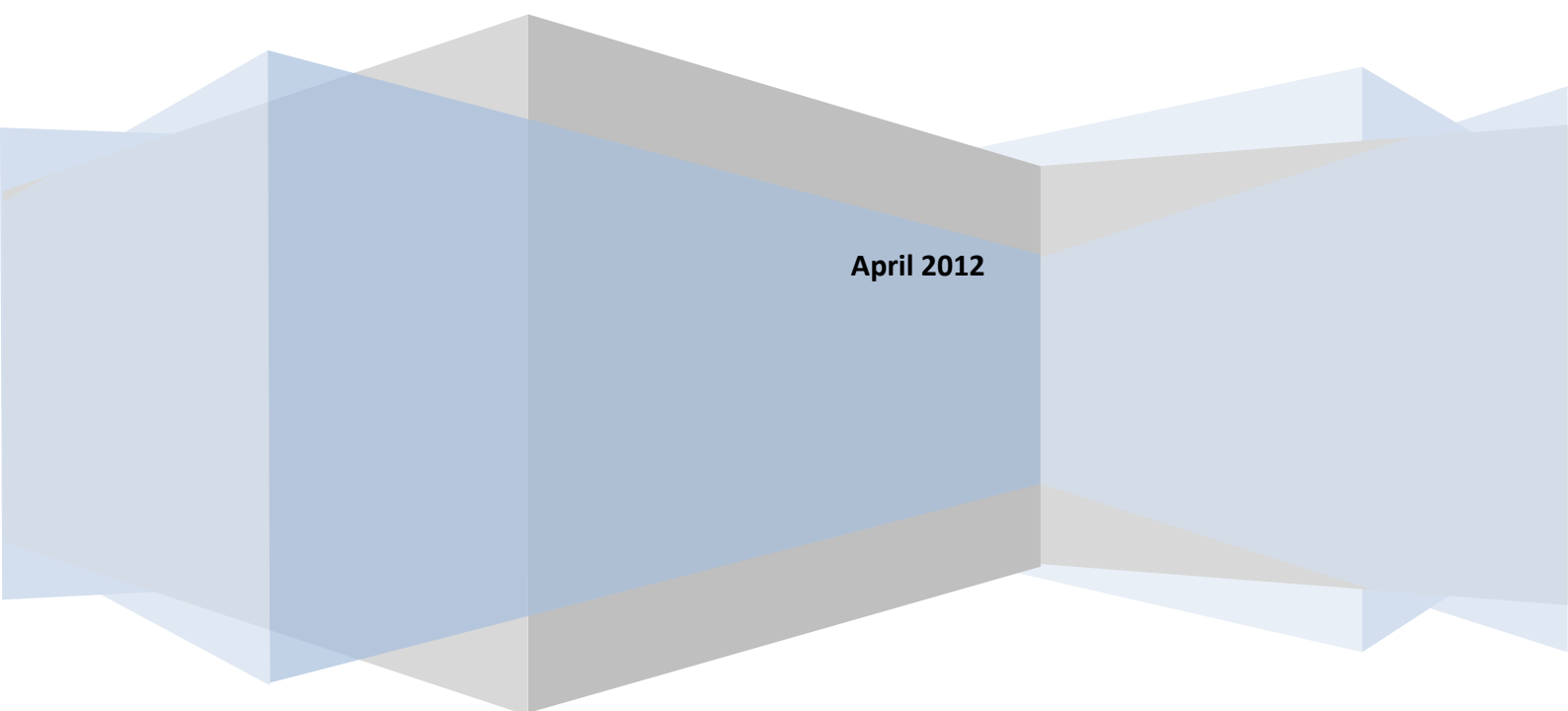


Musculoskeletal Panel

Lecture Notes

James Carter

April 2012

An abstract geometric design at the bottom of the page, consisting of several overlapping, semi-transparent planes in shades of blue and grey, creating a three-dimensional effect.

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Introduction to Musculoskeletal Diseases (Dr Goodfellow, Dr Jones & Dr Hammer)

Diseases of the Musculoskeletal system: Rheumatology

- Rheumatology = prevention, treatment and rehabilitation of MS disorders (including rheumatoid arthritis). NB – soft tissue rheumatism = i.e. CT etc.
- Conditions range from i.e. inflammatory (rheumatoid arthritis), degenerative, back pain and soft tissue.
- Clinical examination will include i.e. history, exam and investigations.
- Inflammatory rheumatic diseases include gout, ankylosing spondylitis etc.
- Some key clinical conditions include: carpal tunnel syndrome, rotator cuff problems, slipped disc, knee problems, hip problems etc. For all of these conditions, consider signs, symptoms and treatment/management.

Signs of Musculoskeletal Problems

- Attitude (i.e. gait).
- Swelling (consider site).
- Muscle wasting.
- Deformity.
- Skin changes.
- Tenderness.
- Reduced movement.
- Increased warmth.
- Muscle strength.
- Stability.
- Function loss.
- Crepitus (cracking noise under skin/joints).

Symptoms of Musculoskeletal Problems

- Pain (site, radiation, worse when i.e. at night etc).
- Stiffness.
- Loss of function.
- Swelling.
- Atrophy.
- Disability (i.e. handicap).
- Systemic illness.
- Sleep disturbance.

Inflammation is a complex response (many mediators) by vascular tissues which protects the organ from harmful stimuli. It is an innate immune response.

Cardinal Signs of Inflammation

- Warmth.
- Redness.
- Swelling.
- Loss of function.
- Pain.

Clinical Conditions

Rheumatoid arthritis is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints.

The process produces an inflammatory response of the synovium (synovitis) secondary to hyperplasia of synovial cells, excess synovial fluid, and the development of pannus in the synovium. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis of the joints. Rheumatoid arthritis can also produce diffuse inflammation in the lungs, pericardium, pleura, and sclera, and also nodular lesions, most common in subcutaneous tissue. Although the cause of rheumatoid arthritis is unknown, autoimmunity plays a pivotal role in both its chronicity and progression, and RA is considered a systemic autoimmune disease.

Women are three times more often affected than men. Onset is most frequent between the ages of 40 and 50, but people of any age can be affected. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated. It is a clinical diagnosis made on the basis of symptoms, physical exam, radiographs (X-rays) and labs. Diagnosis and long-term management are typically performed by a rheumatologist, an expert in joint, muscle and bone diseases.

Osteoarthritis = degenerative arthritis or degenerative joint disease, is a group of mechanical abnormalities involving degradation of joints. Basically everything gets bigger/thicker.

Symptoms may include joint pain, tenderness, stiffness, locking, and sometimes an effusion. A variety of causes, hereditary, developmental, metabolic, and mechanical may initiate processes leading to loss of cartilage. When bone surfaces become less well protected by cartilage, bone may be exposed and damaged. As a result of decreased movement secondary to pain, regional muscles may atrophy, and ligaments may become more lax.

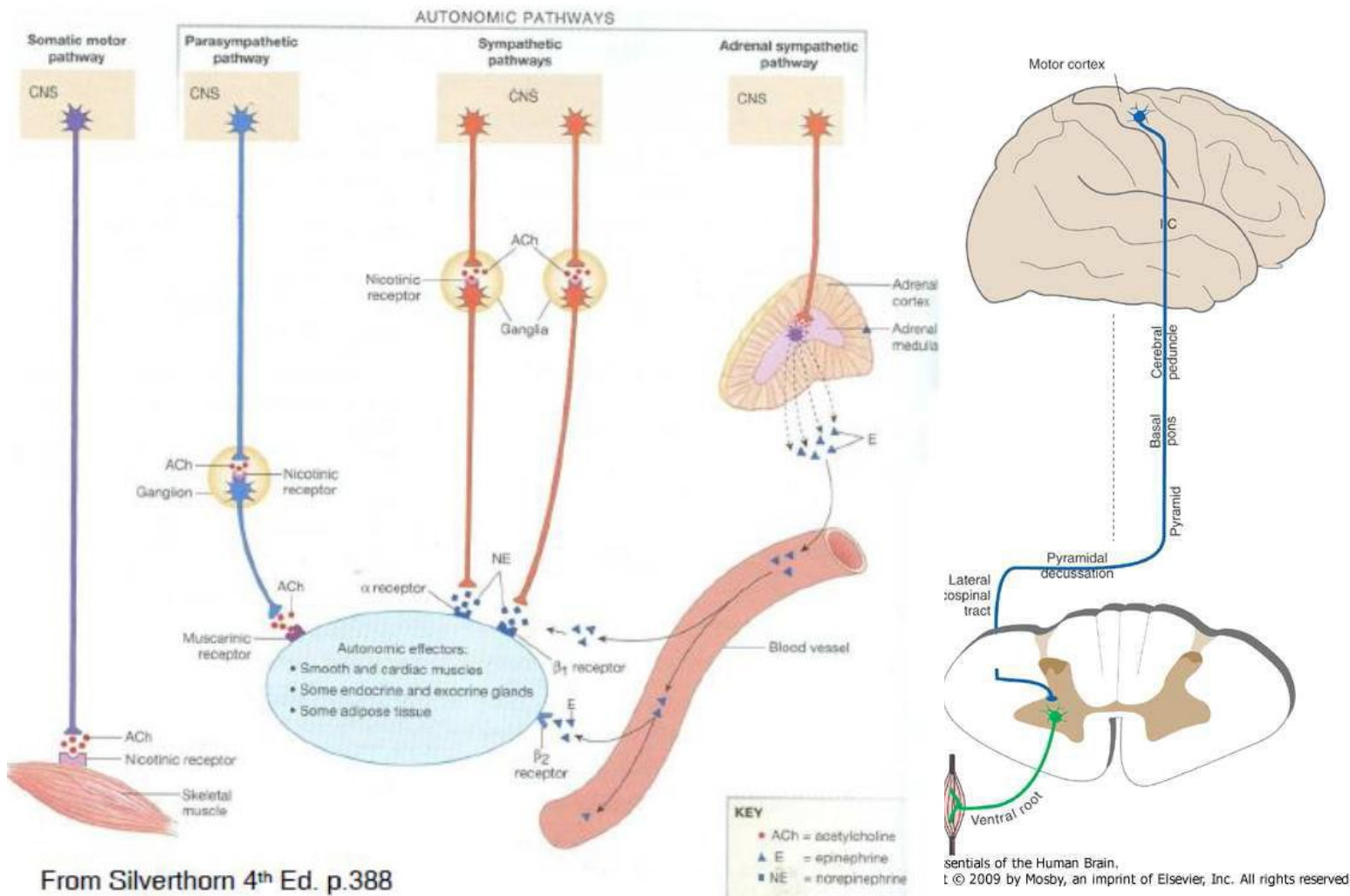
Some investigators believe that mechanical stress on joints underlies all osteoarthritis, with many and varied sources of mechanical stress, including misalignments of bones caused by congenital or pathogenic causes; mechanical injury; overweight; loss of strength in muscles supporting joints; and impairment of peripheral nerves, leading to sudden or uncoordinated movements that overstress joints.

Lower back pain = 65-80% of the population will get this at some point. Many types/causes i.e. inflammatory, mechanical, neurological etc. Clinically must know dermatomes.

Muscle & Muscle Physiology (Dr S Dargan)

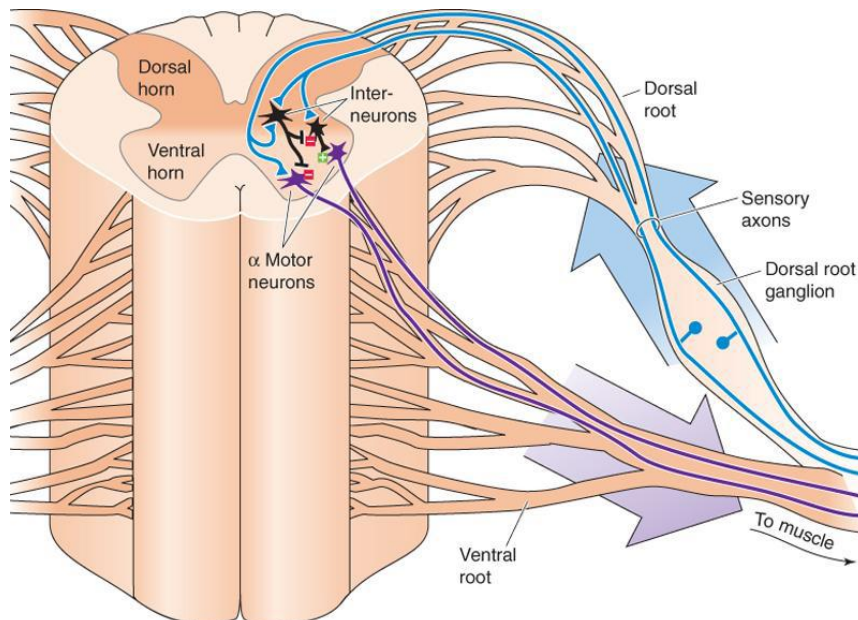
Muscle 1: Innervation of Skeletal Muscle and Neuromuscular Transmission.

Somatic and Autonomic Innervation of Muscle



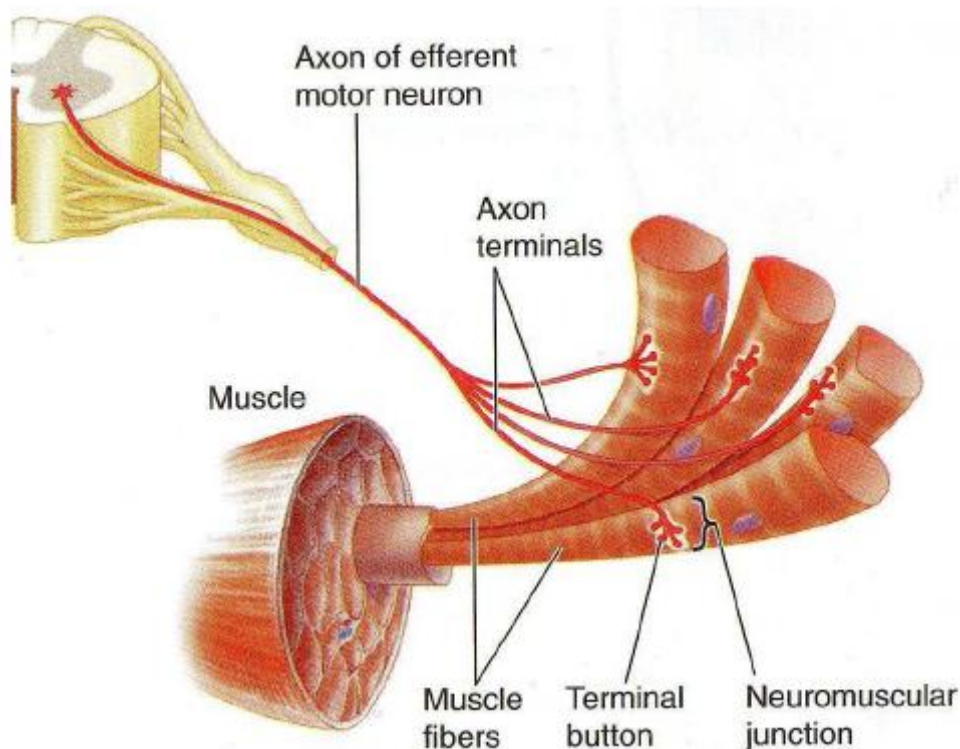
There are three types of muscle: skeletal, smooth and cardiac. Each has its own histology, which is covered in the relevant foundation studies lecture notes.

There are two types of motor neurone: upper and lower. The clinical relevance of these is that they are involved with lower and upper motor neurone disorders and can be utilised in neurological testing. They can result in hypo- and hyper-active reflexes.



Boron & Boulpaep: Medical Physiology, 2nd Edition.
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Nerves innervate skeletal muscle at a neuromuscular junction. These neurones have thick, myelinated axons allowing rapid conduction.

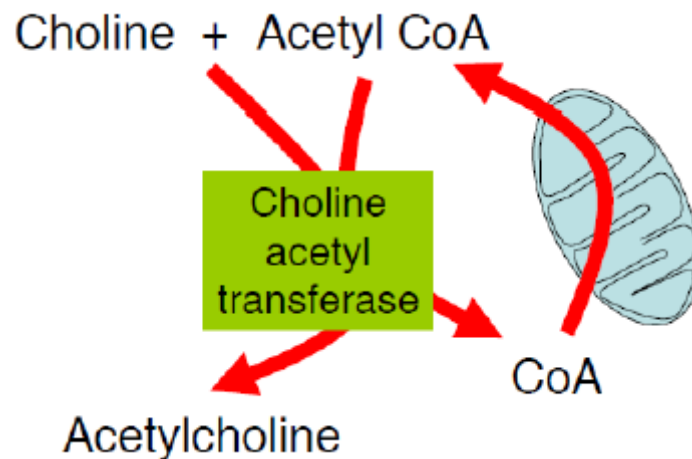


A motor unit is a single α -motor neuron and all of the corresponding muscle fibers it innervates; a motor neurone pool. A Motor neuron pool is the collection of motor neurons that innervate a single skeletal muscle.

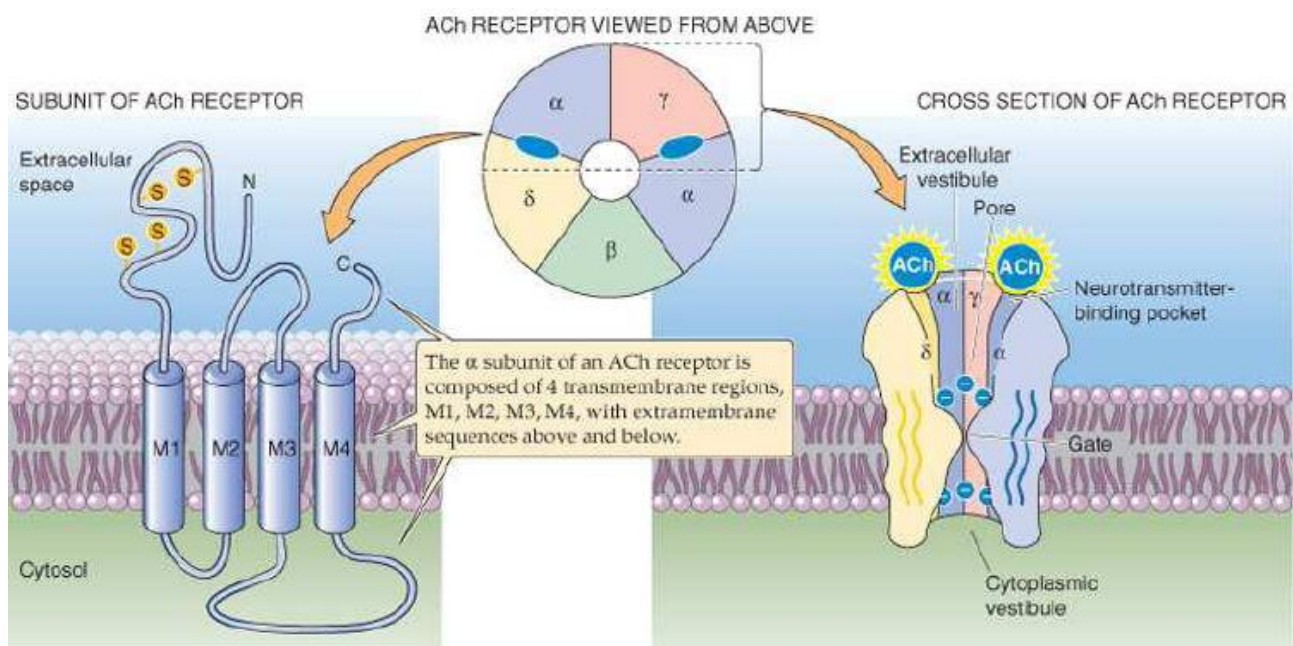
In neuromuscular transmission, an electrical stimulus (action potential) triggers the release of acetylcholine which diffuses across the junctional cleft and binds to specific nicotinic acetylcholine receptors on the muscle cell membrane, activating the entry of sodium which causes a local membrane depolarisation. This triggers an action potential in the muscle fibre.

Acetylcholinesterase degrades the transmitter, terminating the signal (see foundation studies for more details). Under normal conditions, only one muscle fibre action potential is triggered for

each nerve action potential, due to the rapid breakdown of ACh by AChE. Neurones can rapidly synthesise ACh: they never run out, even at high frequency stimulation.



The action of acetylcholine at the motor end plate (ionic basis and receptor type) is shown below.



Muscle 2: Structure of Skeletal Muscle in Relation to Function; Mechanisms of Contraction, Excitation-Contraction Coupling

Structure of a Single Muscle Fibre

In muscle cells, the cell membrane is called the sarcolemma and the cytoplasm is called the sarcoplasm. Muscle fibres are cylindrical and found mainly in the sarcoplasm.

The sarcolemma and sarcoplasmic reticulum form the sarcotubular system.

In a muscle fibre there are lots of myofibrils. Each myofibril has thick and thin filaments, known as myofilaments.

- A bands are dark, thick and made of myosin.
- The H zone is the non-overlapping area between thick and thin filaments.
- I bands are light and contains thin actin filaments.
- Each I band is bisected by a Z line. Between 2 Z lines is a sarcomere.

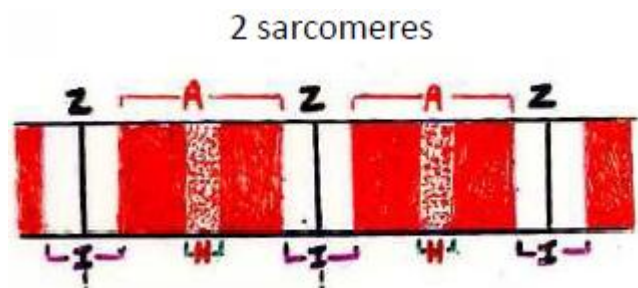
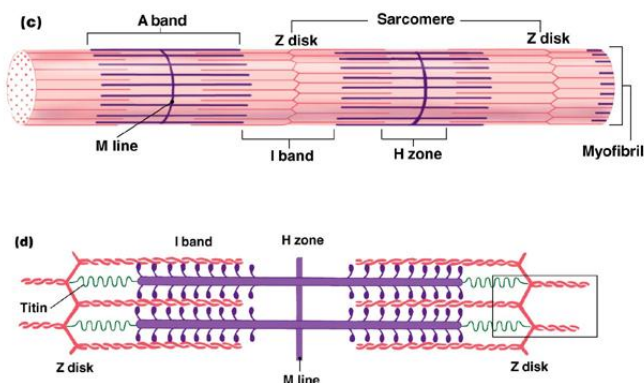
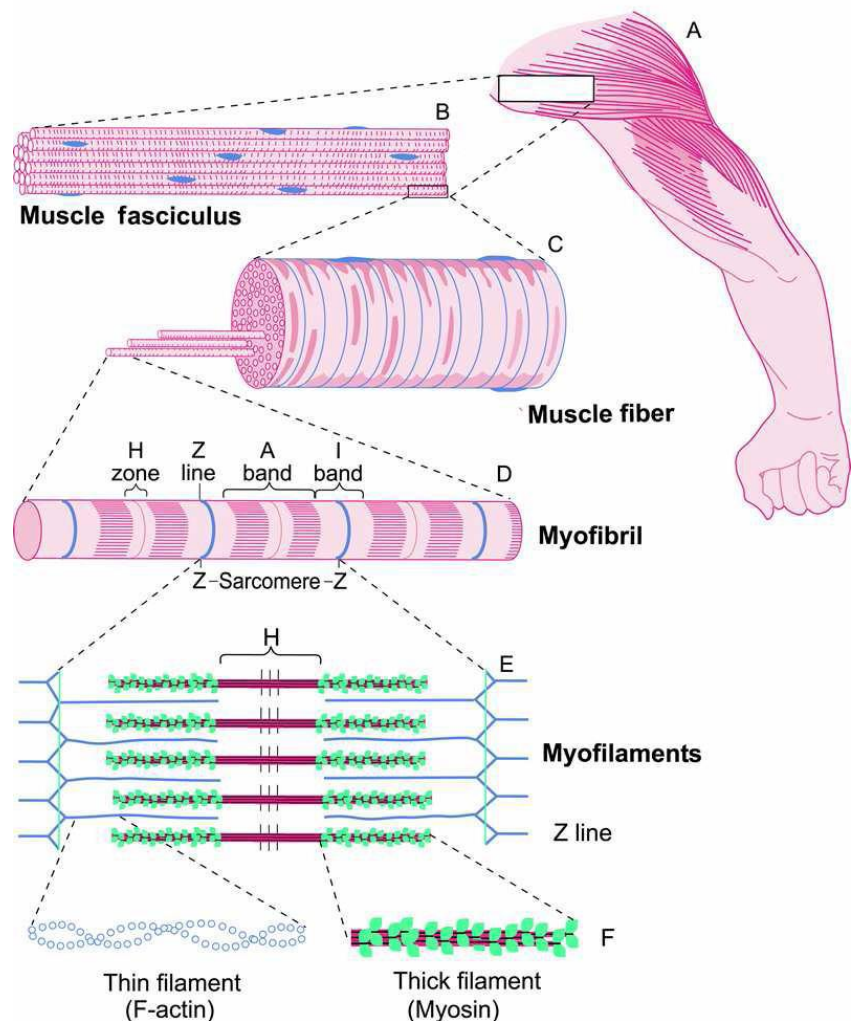


Fig. 12-3

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Thin Actin Filaments ($1\mu\text{m} \times 5\mu\text{m}$)

Thin actin filaments are composed of troponin bound to tropomyosin (regulatory proteins).

Tropomyosin exists as strands, and covers the binding site where actin and myosin join; preventing interaction between them. The core of actin molecules have binding sites for projections from thick/myosin filaments.

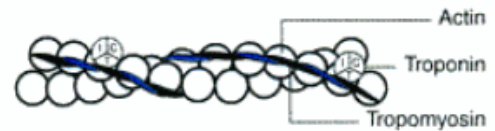
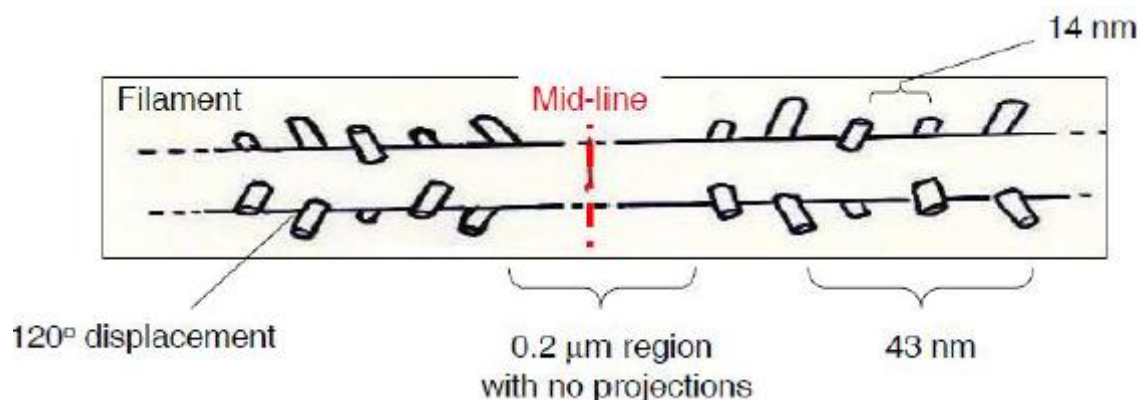
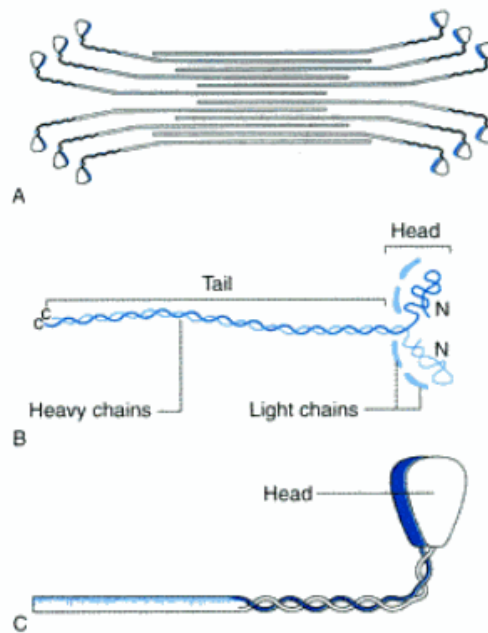


Fig. 2.3-4. Structure of a thin filament.

Thick Myosin Filaments ($1.5\mu\text{m} \times 11\text{nm}$)

Thick myosin filaments are composed of a tail, hinge regions (cleavage sites) and heads (at a 120° displacement).



Thick and thin filaments interact during contraction. This is known as the sliding filament theory.

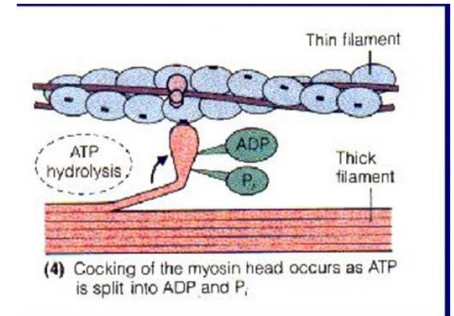
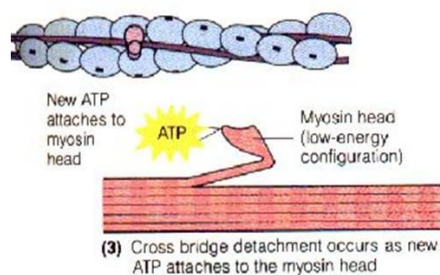
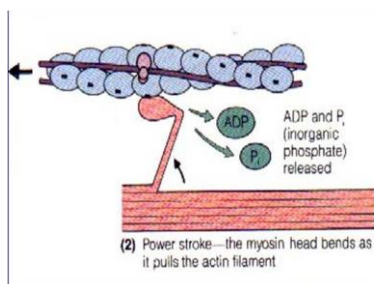
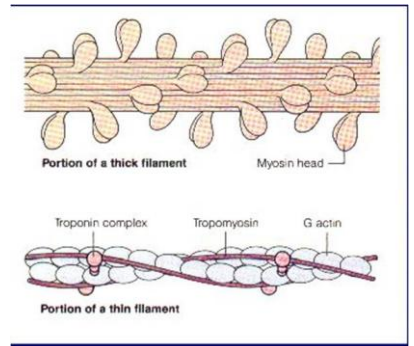
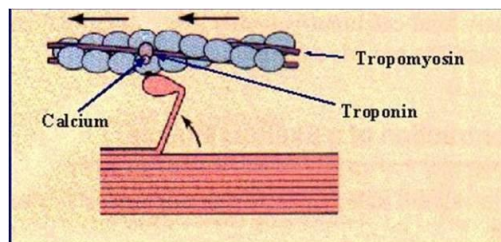
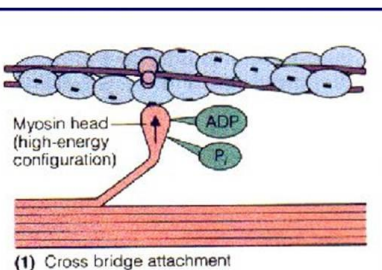
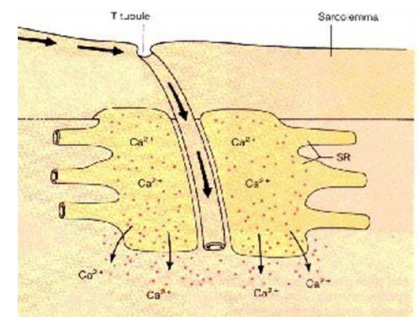
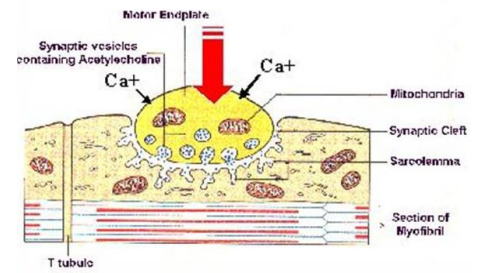


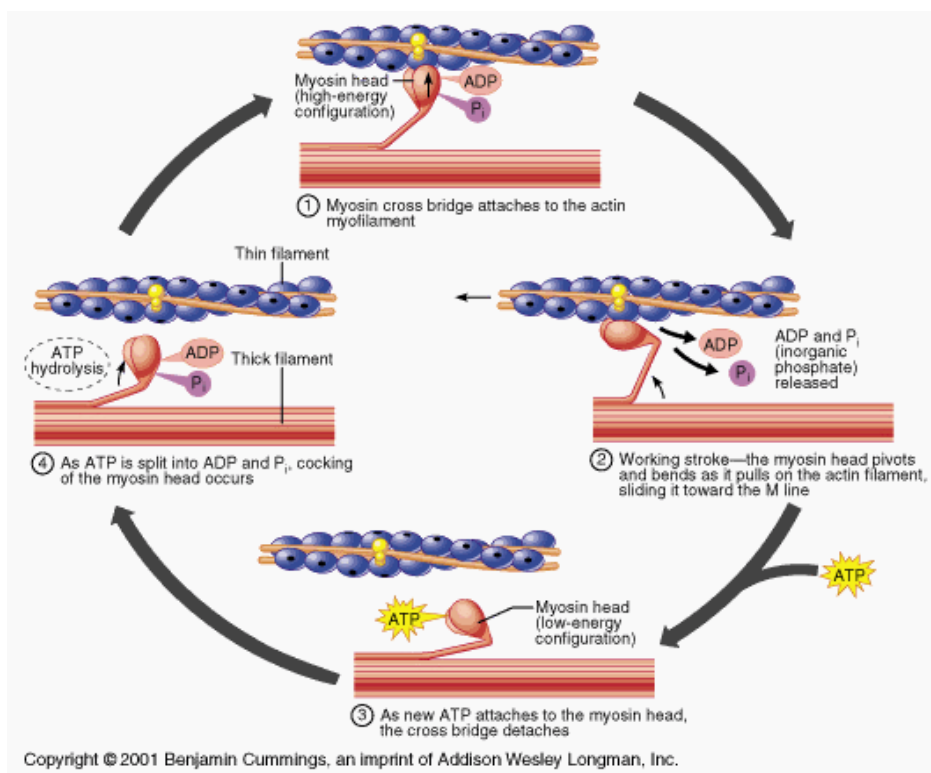
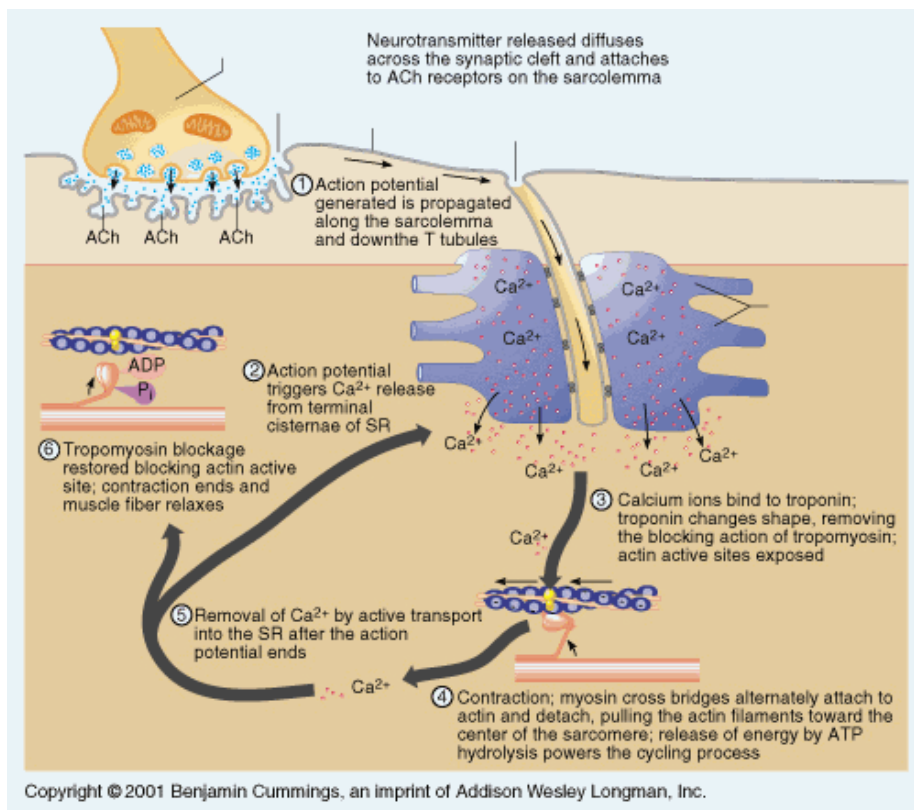
Sliding Filament Theory + Cross Bridge Cycle

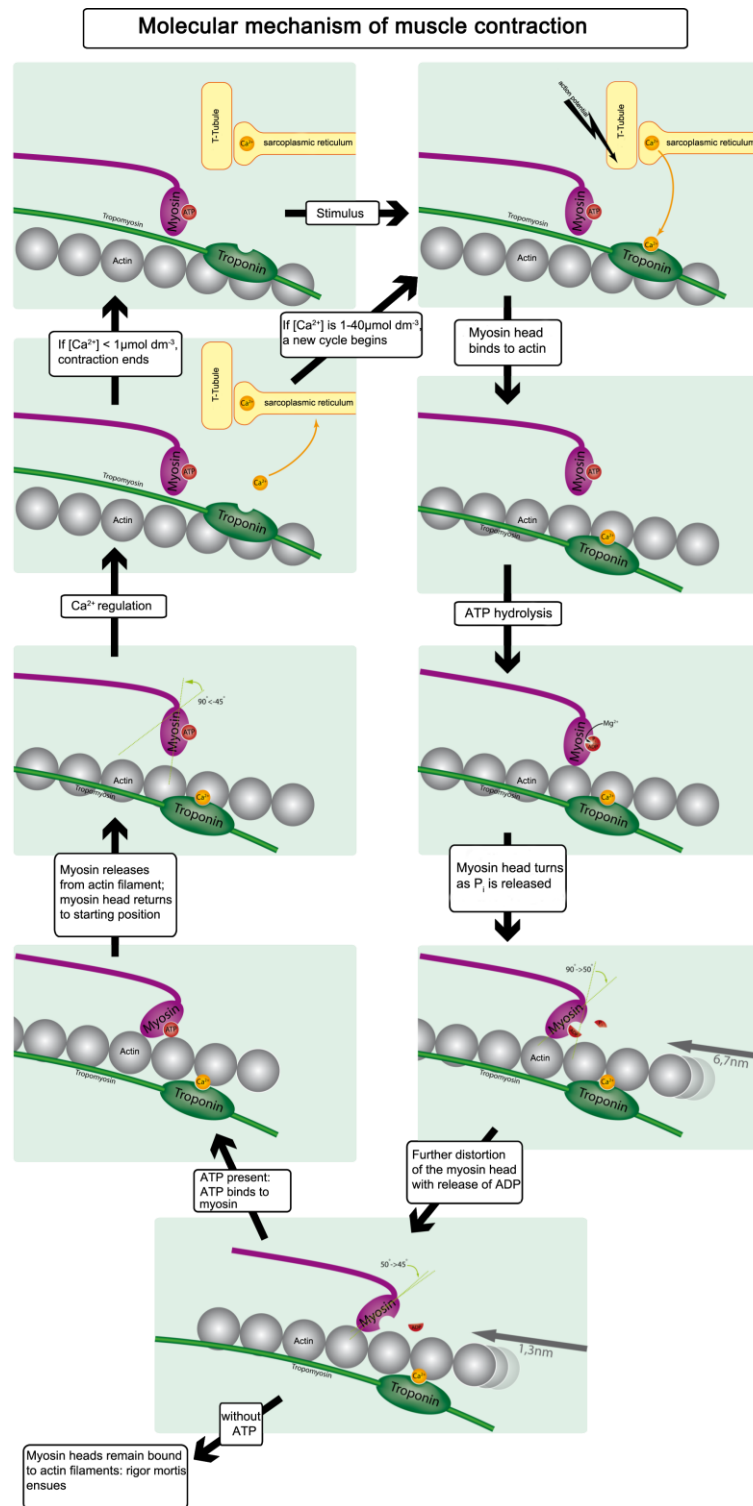
An action potential arrives at a neuromuscular junction. Ca^{2+} is flows into the cell, causing acetylcholine to be released into the synaptic cleft. ACh attaches to receptors on the sarcolemma of the muscle.

The action potential is sent along the sarcolemma, spreading along the muscle fibre. It does this by going deep inside the muscle fibre and down T-tubules.

This triggers the release of Ca^{2+} from the sarcoplasmic reticulum and binds to troponin; troponin changes shape, removing the blocking action of tropomyosin. This exposes actin active sites, allowing myosin heads to attach. Myosin heads attach to actin, forming cross-bridges. Myosin has APTase activity during the power stroke (re-cocking, which requires ATP binding and splitting). Each cycle uses one molecule of ATP. Muscle can synthesise ATP rapidly.





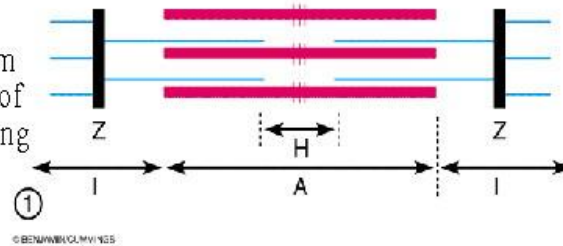


Sarcomeres During Contraction

The lengths of filaments and A bands stay constant. The lengths of I and H bands vary.

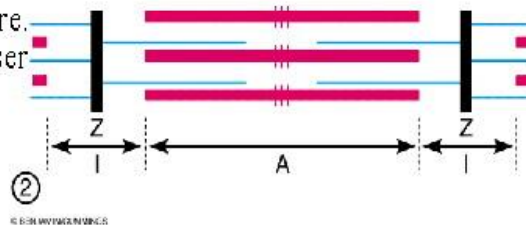
Uncontracted sarcomere.

The Z-lines are at maximum distance apart and overlap of myofilaments is at the resting state



Partially contracted sarcomere.

The Z-lines have moved closer together and the overlap between thin and thick filaments has increased.



Fully contracted sarcomere. The Z-lines are as close together as they can get and the overlap between myofilaments is maximized. Note the overlap between adjacent actin filaments as well as actin and myosin.

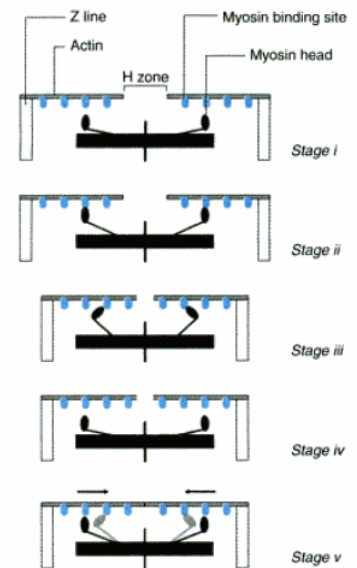
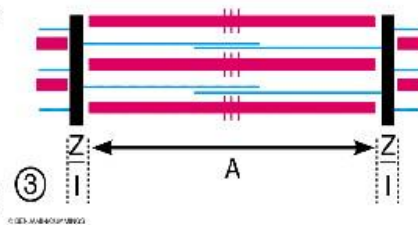
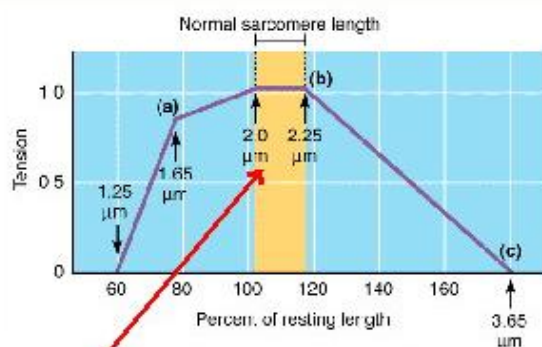


Fig. 2.3-8. Stages of cross-bridge cycling.

The muscle cell gets darker as contraction occurs and the dark A-bands (striations) move closer together and the light I-bands disappear.

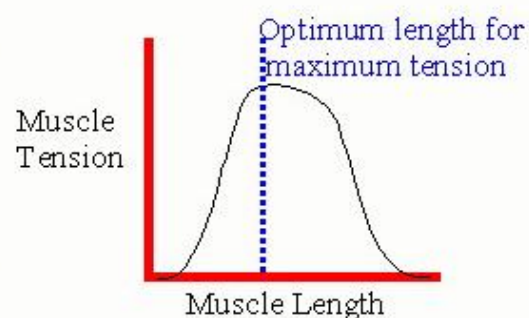
Length-Tension Curve

The Sarcomere



At this length there is maximum overlap of myofilaments producing maximum number of crossbridges and maximum amount of tension.

Whole Muscle



This applies to the entire muscle as well as to individual sarcomeres.

Another way in which the tension of a muscle can vary is due to the length-tension relationship. This relationship expresses the characteristic that within about 10% of the resting length of the muscle, the tension the muscle exerts is at its maximum. At lengths above or below this optimum

length, the tension decreases. In practical terms a muscle will be its strongest at the midpoint in its extensibility.

Note that isometric means that the length of the muscle stays the same (tension increases) and that Isotonic means that the length changes but tension remains the same.

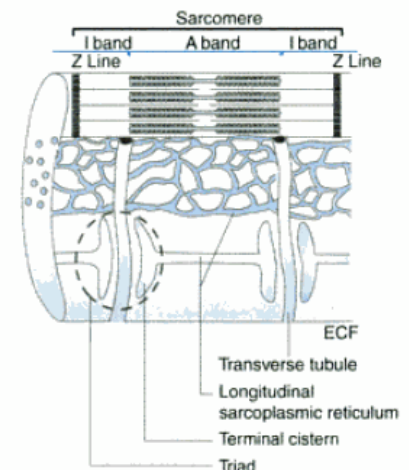
Structure and Function of the Sarcotubular System & Excitation Contraction Coupling

The sarcotubular system conducts depolarisation within a muscle fibre. The components of this are the transverse tubules and the SR (TRIADS).

Figure 2.3-6 - know how to draw a schematic. The key components are: the T tubule; Sarcolemma; SR (i.e. everything within the sarcolemma); longitudinal tubule and terminal cisternae.

Below summarises how an AP in a muscle fibre will lead to cross-bridge activity (excitation-relaxation coupling):

1. AP.
2. Depolarisation of sarcolemma and T tubules.
3. Activation of DHP/L type Ca^{2+} receptors and Ryanodine receptors. Ca^{2+} binds to L type channel.
4. Calcium release from terminal cisternae of SR (causing a rise in intracellular calcium concentration) – can now activate troponin.
5. Contractile machinery activated.
6. Calcium pumped back into SR (longitudinal tubules).
7. Calcium diffuses to terminal cisternae of SR ready to be released again.



3-5. Sarcotubular system showing transverse tubular sarcoplasmic reticulum.

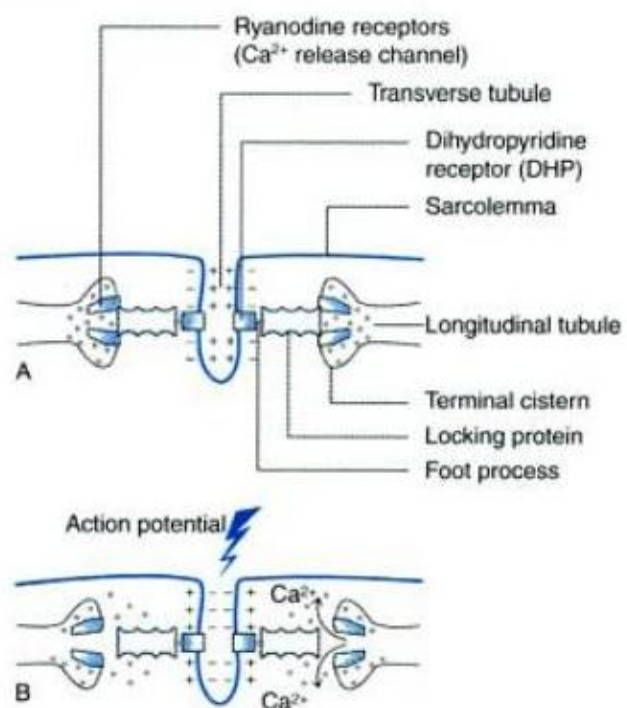
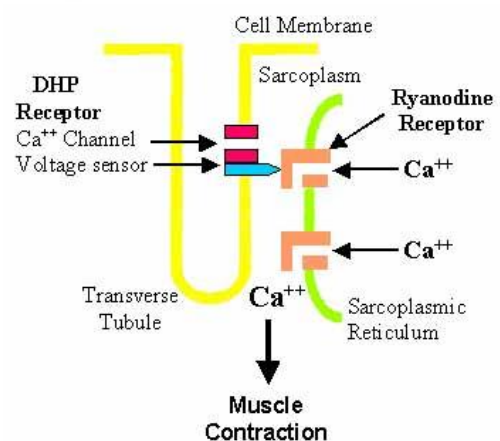


Fig. 2.3-6. Mechanism of release of calcium ions from terminal cistern of longitudinal tubules: A, in resting state Ca^{2+} release channels (RYR) remain closed due to mechanical interlocking between DHP and RYR; and B, during activation state (depolarization of T-tubule). Conformational change in DHP results in opening of RYR and release of Ca^{2+} .



Muscle 3: Summation of Muscle Contractions to give Incomplete and Complete Tetanic Contractions; Types of Muscle Contraction

Time Course of Activation Following a Single Stimulus: Relation to Sarcoplasmic Free Ca^{2+} Concentration

The contractile machinery is dependent upon calcium concentration.

Following an AP (rapid spike), Ca^{2+} concentration is high (due to a rapid increase). It is released from the SR (DHP and Ryanodine receptors are involved).

Following contraction, Ca^{2+} concentration decreases as Ca^{2+} enters the SR via ATPase.

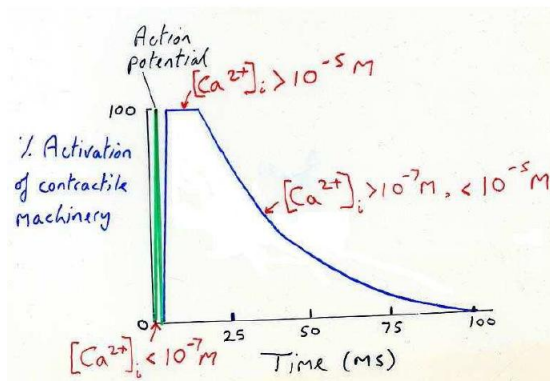
Rate dependent upon temperature because enzymes and ATPase (aka SERCA), are responsible for bringing calcium back into the SR.

As calcium concentration increases, the muscle contracts (as the contractile machinery has been activated).

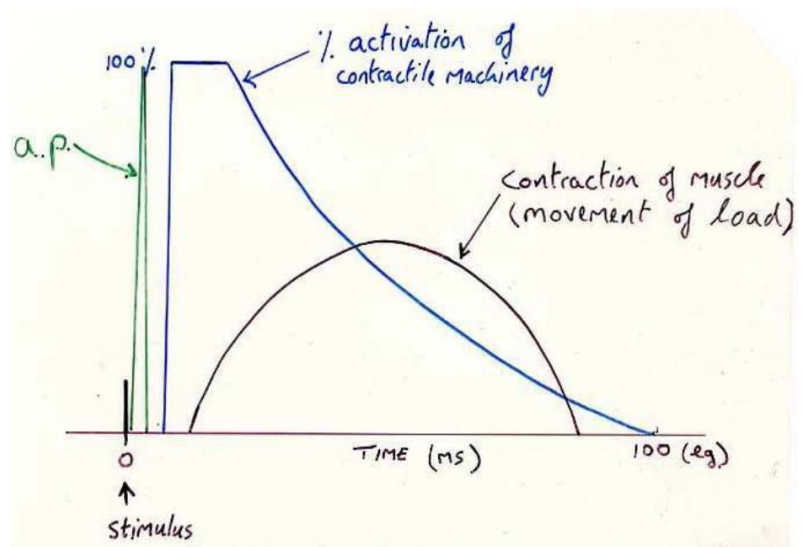
As calcium concentration decreases, the muscle relaxes (a lesser percentage of the contractile machinery is now active).

Delays are present in the process due to the molecular events/steps that are taking place.

Time-course of activation following single stimulus, and relation to sarcoplasmic free Ca^{2+} concentration

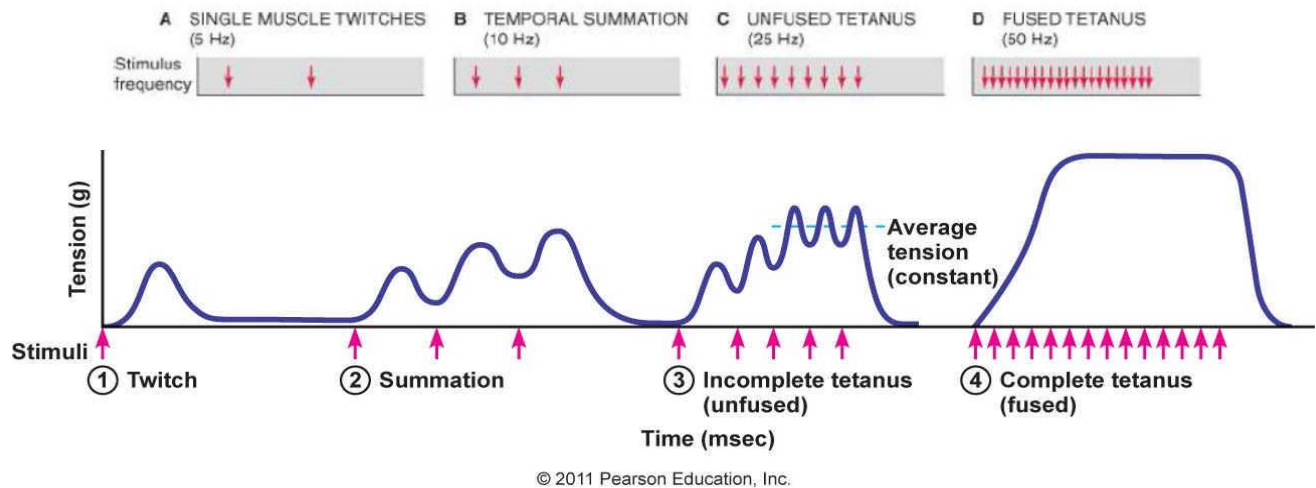


* Rate is highly temperature dependent (decrease X2 for a 10°C fall in temp)
Thus rate of relaxation of muscle decreases as temperature decreases



Tetanic Fusion Frequency & Temperature Effects

Different frequencies of stimuli lead to different results; the frequency is increased until tetanic fusion occurs (TFF). TFF is typically between 25-50Hz. TFF varies between different muscles (in humans, it ranges between 15-200/sec) and with temperature (TFF decreases as temperature decreases).



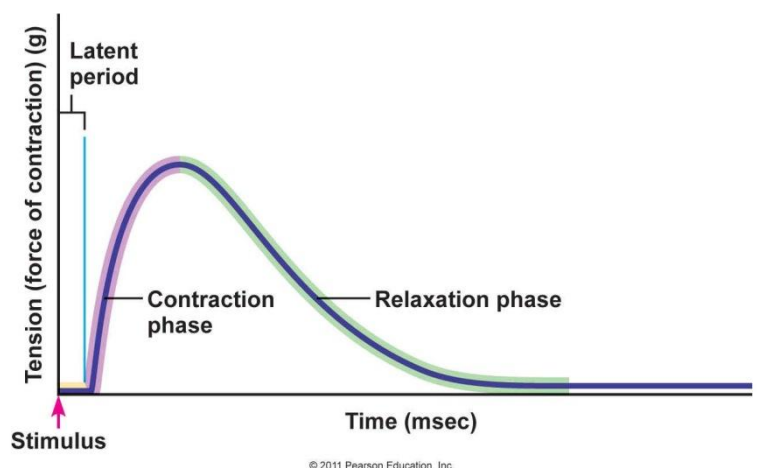
Note that with repeated stimuli, summation of responses occurs leading to incomplete and then complete tetani.

A twitch is the mechanical response of a single muscle fibre to a single action potential. Typically, these last between 7 and 100ms.

Latent period is the delay (of a few milliseconds) between an action potential and the start of a contraction, and reflects the time for excitation-contraction coupling.

During the contraction phase, cytosolic calcium levels are increasing as released calcium exceeds uptake.

During the relaxation phase, cytosolic calcium is decreasing as reuptake exceeds release.



A myogram can measure twitch tension development.

Note that rate is temperature dependent: the rate of relaxation of muscle decreases as temperature decreases.

As a muscle twitch is fairly slow compared to an action potential, many action potentials can arrive before a single twitch is completed. This causes the twitches to bunch up and results in the generation of a force that is greater than a single twitch. This process is called temporal summation and increases the strength of signals in each fiber. When the frequency of stimulation is so high that Ca^{2+} levels rise to peak levels, summation results in the level of tension reaching a plateau called tetanus. This can be either unfused or fused.

Unfused (or incomplete) tetanus is when the frequency of stimuli is high enough to cause tetanus but the tension oscillates around an average level.

At greater frequencies of stimulus, levels of Ca^{2+} peak and cause a maximum number of cross-bridges to cycle. At this point, a tension plateau forms and the tetanus is called fused (or complete).

Note that frequency: is measured in Hz and represents cycles per second, and therefore the number of APs per second.

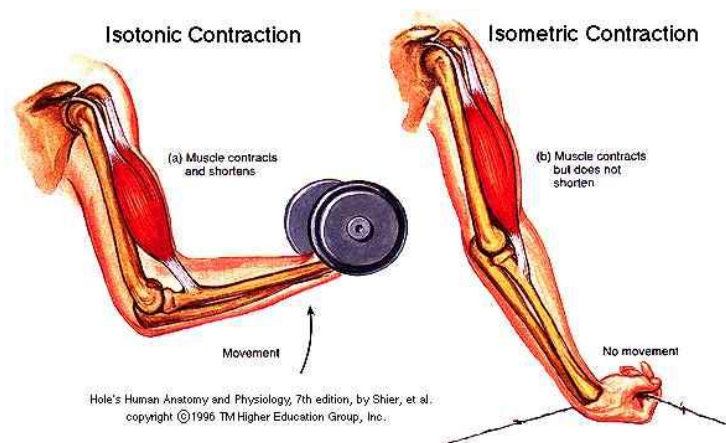
- 10Hz – 10 AP per sec – 1 sec = 1000ms, $1000/10 = 100$. So 1 AP every 100ms.
- For 100Hz – 100 AP per sec – 1 sec = 1000ms, $1000/100 = 10$. So 1 AP every 10ms.

Isotonic and Isometric Contractions

Isometric contraction is where the muscle fibre remains at a constant length despite changes in tension. An example would be when, trying to lift an item which is too heavy: the arm muscles contract, but there is no resultant movement.

Isotonic contraction is where the muscle shortens against a load. For example, lifting a heavy load held at arm's length, or rowing a boat.

Note that in exercise, combinations of the two contractions can be used. For example, the bench press exercise uses an isotonic contraction to lift the weight, and an isometric contraction whilst holding it up.



Muscle 4: Muscle Fibre Types, Activation of Motor Units during Voluntary Contractions, Gradation of Contractions

Different Types of Fibres

Different fibre types have different properties (speed of contraction and metabolic characteristics) as well as physiological classification (dependent on the speed of contraction and their resistance to fatigue).

Contraction time is the time from the beginning to the peak of the twitch. Speed of contraction is determined by the rate of ATP splitting (ATP determines rate of cross bridge cycling and thus rate at which filaments slide) and the actomyosin complex (ATPase activity), which is determined by the properties of myosin.

There are three types of muscle fibre types:

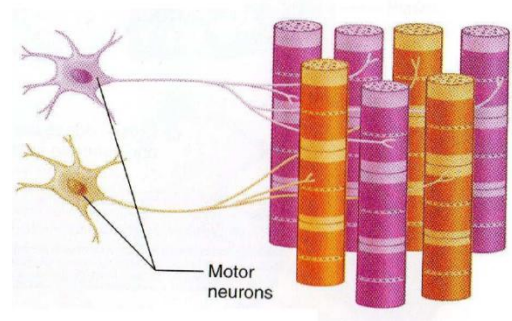
1. Slow twitch. Type 1.
2. Fast twitch fatigue resistant. Type 2a.
3. Fast twitch fatigable. Type 2b.

In fast twitch fibres – myosin causes high rate of ATP splitting. Therefore, there is a high rate of ATP usage. In slow twitch fibres myosin which causes a low rate of ATP splitting is present. Therefore, ATP is not used up as quickly. The contraction time of slow twitch fibres is 100ms; the contraction time of fast twitch fibres is about 7.5 to 40ms.

Most human muscles contain a mixture of all 3 types of fibres; relative proportions vary between muscles. All the fibres in an individual motor unit (1 motor neuron that supplies several fibres) are of the same type.

The number of muscle fibres per motor unit varies:

Muscles involved with fine movement have few motor units (some as few as 5). Postural muscles have large motor units (some more than 1500).



▲ **TABLE 8-1**

Characteristics of Skeletal Muscle Fibers

Summary

CHARACTERISTIC	TYPE OF FIBER		
	Slow-Oxidative (Type I)	Fast-Oxidative (Type IIa)	Fast-Glycolytic (Type IIx)
Myosin-ATPase Activity	Low	High	High
Speed of Contraction	Slow	Fast	Fast
Resistance to Fatigue	High	Intermediate	Low
Oxidative Phosphorylation Capacity	High	High	Low
Enzymes for Anaerobic Glycolysis	Low	Intermediate	High
Mitochondria	Many	Many	Few
Capillaries	Many	Many	Few
Myoglobin Content	High	High	Low
Color of Fiber	Red	Red	White
Glycogen Content	Low	Intermediate	High

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Table from Sherwood's Human Physiology 7th ed. p280

Know all of the key aspects of this table.

Consider that:

- Oxidative phosphorylation is linked to mitochondria.
- Myoglobin content is linked to the colour of the fibre.
- Enzymes for anaerobic glycolysis are linked to glycogen content.
- Entries 1, 2 and 3 also linked.

Examples of different types of muscles include postural muscles (which maintain activity for long periods without fatigue and have a high proportion of slow twitch fibres); and biceps brachii (which may be called upon to produce large amounts of tension rapidly and has a larger proportion of the fast-twitch fatigable fibres [larger motor units]).

Exercise and Fibre Types

The proportion of fibre types is not rigidly fixed (experiments have shown this). The pattern of neuronal input is the key factor: for example cross innervating muscles has shown that muscles will slowly change their metabolic and contractile characteristics (fast twitch muscle becomes slow and vice versa).

Exercise and Training in Humans

Exercise and training can result in changes in muscle fibre types. Endurance training such as long distance events cause fast twitch fatigable fibres to become fatigue resistant fibres. These changes

occur in the muscles that are predominantly used. High intensity short duration training such as sprinting can lead to hypertrophy of fast twitch fatigable fibres (synthesis of actin and myosin and therefore a large increase in diameter).

Voluntary Contractions

Voluntary contractions are where motor units are activated and fire out of phase. The strength of contraction required determines the firing rate. Strong contractions are a result of increased voluntary effort: more motor units are activated (motor unit summation) and activated at higher frequencies (wave summation). Weak contractions are a result of only a few motor units firing (small motor units activated). These become activated at low frequencies.

Sustained voluntary contraction (sub maximal) comes as a result of increased voluntary effort: more motor units are recruited, more motor units are activated at higher frequencies, and the strength of contraction can be maintained for a while.

Fatigue is where some muscles become incapable of contraction. The physiological definition states that fatigue is the awareness that increasing amount of effort is required to maintain the same degree of performance. Experiments have shown that: with supreme effort it is possible to activate all of the motor units at such a frequency to give a complete tetanus.

Larger muscles are different. Peak activation is not normally achieved by voluntary effort as it is limited by a more difficult activation of the motor neurone pool to the muscle. The extent to which the muscle can be activated varies between individuals and within individuals at different times. Muscle tension is influenced by the degree of motivation. NB – shouting = motivation = increases muscle tension.

Muscle 5: Fatigue in Muscle Contractions

Delayed Onset Muscle Soreness (DOMS)

DOMS is muscle pain which presents 8-12 hours after exercise. It is caused by damage to muscle fibres, such as bleeding, inflammation and disruption of filaments near Z lines. Elevated blood CPK (creatine phosphokinase) levels are also present.

ATP Sources: Stores

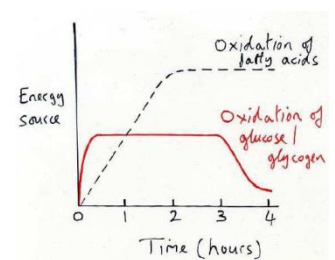
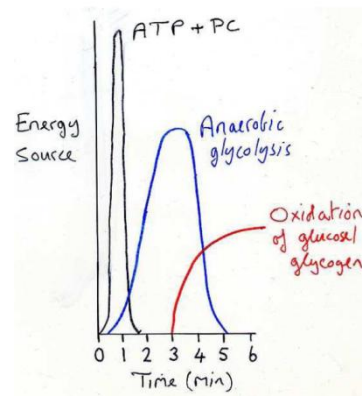
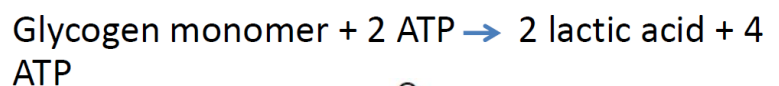
Muscles contain small stores of ATP, enough only for a few twitches to occur. ATP must be synthesised to keep pace with utilisation. In muscles, ATP is required for contraction in cross bridge cycling and sliding filaments (in relaxation, by pumping calcium back into the SR).

Creatine Phosphate (Phosphocreatine)

Creatine phosphate is present in muscles in high concentrations (around 15-20mM). There is six times more creatine phosphate than ATP. The phosphate group is transferred to ADP to form ATP ($CP + ADP + H = ATP + \text{creatine}$). Dephosphorylation of CP provides a rapid source of ATP. The creatine phosphate stores are exhausted in 15 seconds of exercise.

Glycolysis (Anaerobic)

Muscle contains large amounts of glycogen, which is an important source of ATP during heavy exercise. It has a poor efficiency in comparison to oxidative metabolism, producing only 2 ATP per molecule of glucose. Large amounts of lactic acid build up (note that H^+ also accumulates).



Oxidative Phosphorylation (Aerobic)

Oxidative phosphorylation has a high yield of ATP. Sources for this process are free fatty acids and glucose.

Appreciate that these four processes occur at different times.

Fatigue

Fatigue is defined as “an exercise-induced reduction in the ability of a muscle to generate force or power”. It is well known that the effort required to sustain a voluntary contraction increases and strength of contraction declines as fatigue occurs.

Central and Peripheral Fatigue

Central fatigue is due to changes in the CNS (activation of motor neurones falls off) and peripheral fatigue is due to changes in the muscle itself. Experimentally, the process appears to be peripheral (in well-motivated subjects), however the results are inconclusive overall.

Peripheral fatigue

Peripheral fatigue is due to biochemical change in muscle, such as accumulation of metabolites. Examples include H^+ (from hydrolysis of ATP and anaerobic glycolysis), H_2PO_4 (from hydrolysis of ATP and breakdown of CP), and ADP or AMP.

Fatigue is associated with failure of contractile machinery and impairment of calcium release from the SR. Fatigue is more likely in strong maintained contractions because the blood supply to muscle is reduced due to compression of blood vessels in the muscle. This means that metabolites are not washed away in blood stream, which can cause pain (by stimulating sensory nerve endings).

Central Fatigue

Central fatigue is present due to complex movements involving large muscles or groups of muscles. The strength of contraction falls off before muscle fibres themselves become incapable of full contraction. This is due to failure of activation of motor neurones due to failure to generate an action potential or transmission at some level in the complex motor pathways controlling movement.

Central fatigue is a complex process and not much is known about its mechanisms. They may involve sensory stimuli arriving in CNS which contribute to the inhibition of motor neurone activation. It is not known at which level this occurs in the motor control system, but it is probably widespread, occurring at cortical and spinal levels.

Motivation and Central Fatigue

Central fatigue involves psychological and other poorly explainable factors. It is a variable phenomenon (between and within individuals). Motivation, encouragement and reward play a role (a task can be performed better with encouragement).

Notes:

- Isotonic contraction/concentric contraction: muscle shortens against a load.
- Isometric contraction: muscle held at constant length but tension changes.
- Eccentric contraction: muscle is being extended by a load but is resisting extension; muscle is lengthening at a controlled rate against the load.

Clinical relevance: NM disorders such as MS and MG (fatigue with muscle weakness); glycogen storage diseases; cachexia (involuntary weight loss, muscle wasting etc.); and chronic fatigue syndrome (which can present with muscle pain, joint pain and headache).

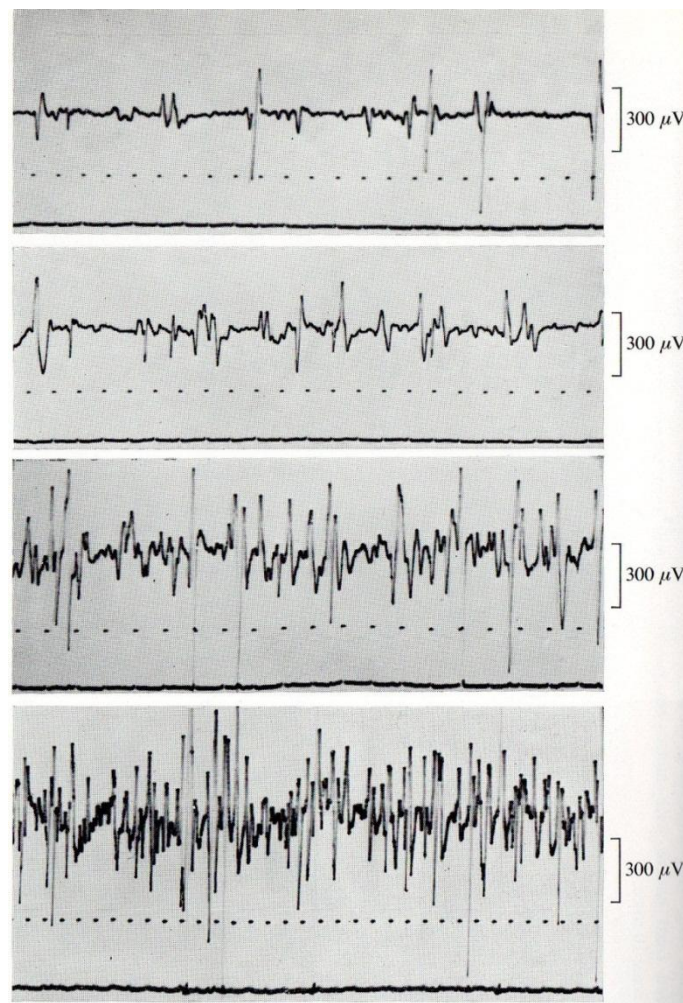
Muscle 6: Disorders of Motor Function: Investigation by Electromyography; Levels at which Damage or Disease could affect Muscular Function

Electromyography (EMG)

Electromyography records changes in extracellular potential arising from action potentials in muscle fibres. It is an extracellular recording technique utilising surface electrodes (pads placed on the skin) and intramuscular electrodes (needles).

In this way, we can measure motor unit potentials (the summed electrical activity from the discharge of all fibres in a motor unit). These are usually biphasic and have a size (amplitude) related to the number of muscle fibres firing simultaneously.

In normal muscle, when relaxed there is no signal (due to no action potentials). A weak voluntary contraction will result in small spikes. We never see discharge of single muscle fibres. The frequency of potentials increases as the subject increases the strength of contraction. This is because more motor units are recruited, each unit firing more and more frequently. The spikes eventually start to merge and become “fuzzy”. The changes in voluntary contraction are shown below.



We can stimulate the nerve to a muscle electrically. We see a large biphasic spike in response to each stimulus, as an action potential is fired more or less simultaneously in each fibre of the muscle.

Acute Denervation

Wallerian degeneration is the process which results when a motor nerve to a muscle is cut or crushed (due to trauma). The nerve can no longer conduct action potential, and the muscle becomes paralysed. 2-3 weeks after the trauma, the muscle fibres become hyper-excitable and individual fibres begin to contract spontaneously (fibrillation potentials). Fibrillation potentials are a sign of acute denervation. They cannot be detected visibly but can be detected using intramuscular EMG.

Atrophy

If the nerve does not regenerate, progressive atrophy of muscle fibres occurs. This is a slow and variable process in humans which can take up to two years. The muscle fibres shrink, become less sensitive to electrical stimulation and eventually are lost and replaced by fatty and fibrous material.

Regeneration

Some cut axons can grow to reinnervate muscle. Growth occurs down the endoneurial tube in the distal part of the severed nerve at a rate of 3-4mm a day. This is most likely to occur if the nerve was severed cleanly and the severed ends are brought together surgically and sutured (or glued). Some of the re-grown axons reach muscle where they branch and reinnervate muscle fibres. This ultimately results in a relatively small number of large motor units which give a reasonable recovery of function. However, fine control remains diminished.

Chronic Partial Denervation

As motor neuron cell bodies or axons become damaged, they tend to fire action potentials spontaneously. This results in fasciculation of muscle (the random discharge of individual motor units). This occurs in certain diseases, such as poliomyelitis (where anterior horn cells are destroyed) or peripheral neuritis (damage to the axons of motor neurons).

The discharge of large motor units is seen as small rippling movements under the skin. EMG is able to observe these random spikes whilst the muscle is at rest (they are bigger than fibrillation potentials due to whole motor units firing).

Sprouting (the process whereby a neuron generates additional branches to establish new links between existing neurons) of the surviving motor neurones reinnervates muscle fibres that had lost their supply. This results in the remaining motor units in the muscle gradually increasing in size (leading to giant motor unit potentials).

Giant Motor Unit Potentials

These giant motor potentials means that the discharge of each motor neurone simultaneously activates many muscle fibres: this helps to maintain the strength of the muscle to some extent; however, the movements are coarsened due to a decrease in the number of motor units. Note

that giant motor unit potentials can also occur following regeneration of a nerve to a muscle that was acutely denervated.

Muscle Disorders

Potentially, damage or disease could affect muscular function at any of the following levels:

- Motor control centres in the CNS
- Descending pathways from motor control centres
- Motor neurone cell body
- Motor neurone axon
- Neuromuscular junction
- Muscle fibres

Upper or lower motor neurone lesions and myopathies may also result. Upper motor neuron lesions lead to impairment of voluntary activity, exaggerated spinal reflexes, but no muscle atrophy (usually). Lower motor neurone lesions result in the impairment of voluntary activity and reflex activity, as well as muscle atrophy. Some disorders, such as motor neurone disease, may involve both upper and lower motor neurone lesions.

Myasthenia gravis is an autoimmune disease associated with a decreased number of acetylcholine receptors at the motor end plate. Muscle weakness and fatigue results and is associated with the failure of neuromuscular transmission. It is diagnosed by anticholinesterases and treated via removal of the thymus.

Poliomyelitis is associated with the destruction of motor neurone cell bodies and results in impairment of both voluntary and reflex activity, as well as muscle atrophy.

Dystrophin is caused by an X-linked recessive gene. When the dystrophin gene is absent or abnormal, membranes become leaky to Ca^{2+} , proteolytic enzymes are activated, and the contractile machinery is destroyed.

General Symptoms of patients suffering from a disorder affecting motor units include weakness and rapid onset of fatigue.

Muscle 7: Smooth muscle

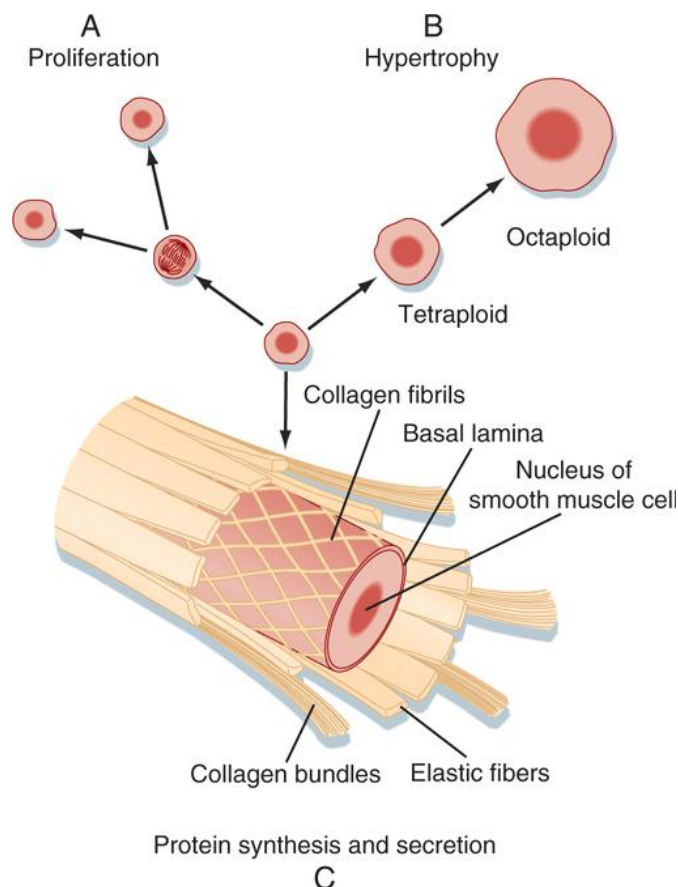
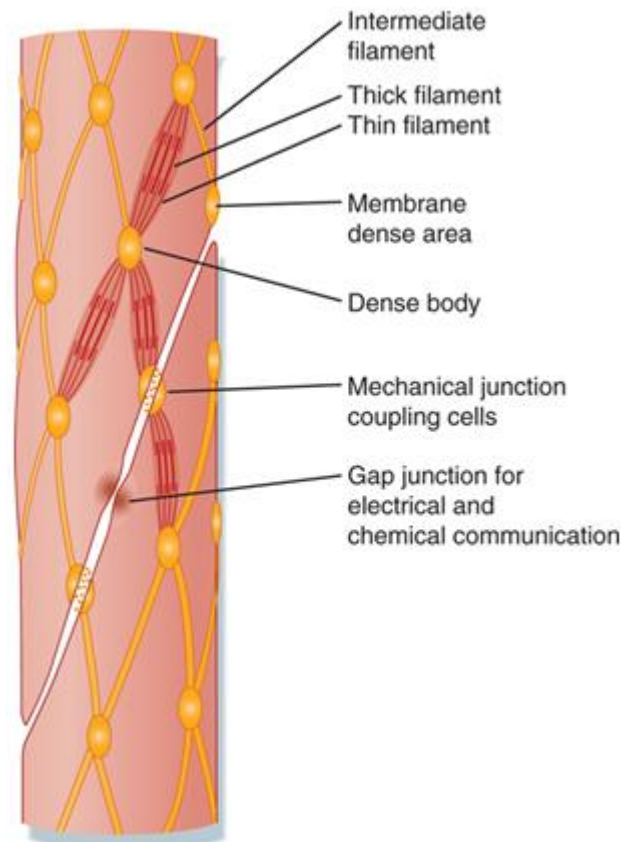
Examples

Smooth muscle is located in internal organs (viscera); the walls of blood vessels; around hollow organs (such as the urinary bladder); and in layers around the respiratory, circulatory, digestive and reproductive tracts. The function of smooth muscle is in moving food, urine and reproductive tract secretions; controlling the diameter of respiratory passageways; and in regulating the diameter of blood vessels.

Structure

Smooth muscle consists of spindle-shaped cells which are smaller than skeletal muscle cells (measuring 100-300µm in length and 2-5µm in width). They have only a single nucleus. Smooth muscle fibres are often embedded in a matrix of connective tissue and are arranged in series and in parallel with one another.

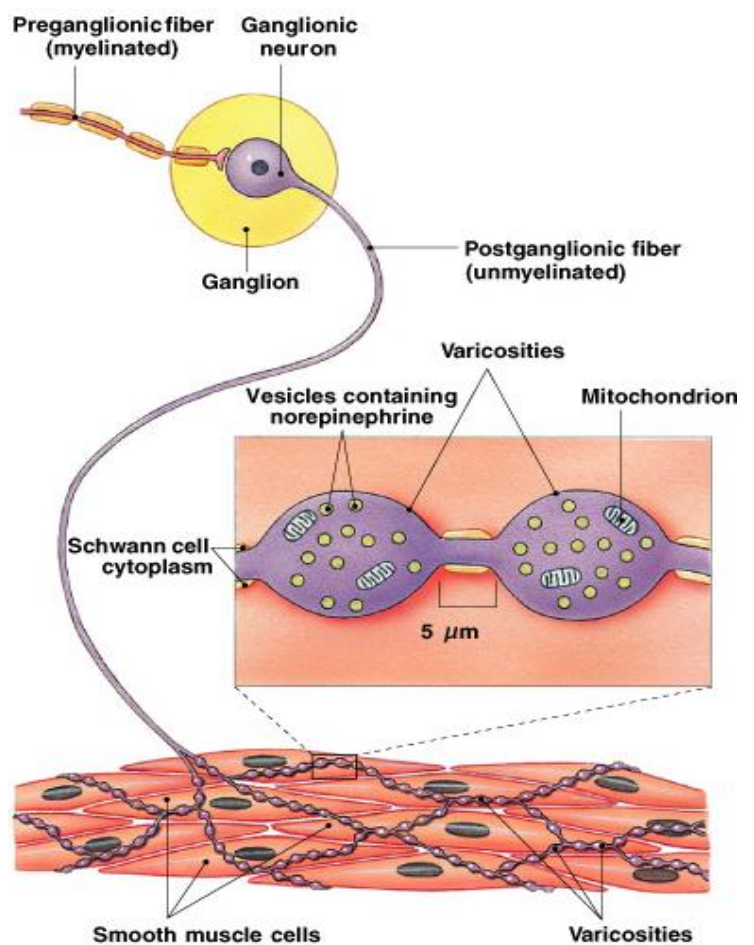
Smooth muscle retains the capacity to divide during normal growth and pathology. Cells can hypertrophy with increased load, and can synthesize and secrete the constituents of the extracellular matrix.



Innervation of Smooth Muscle

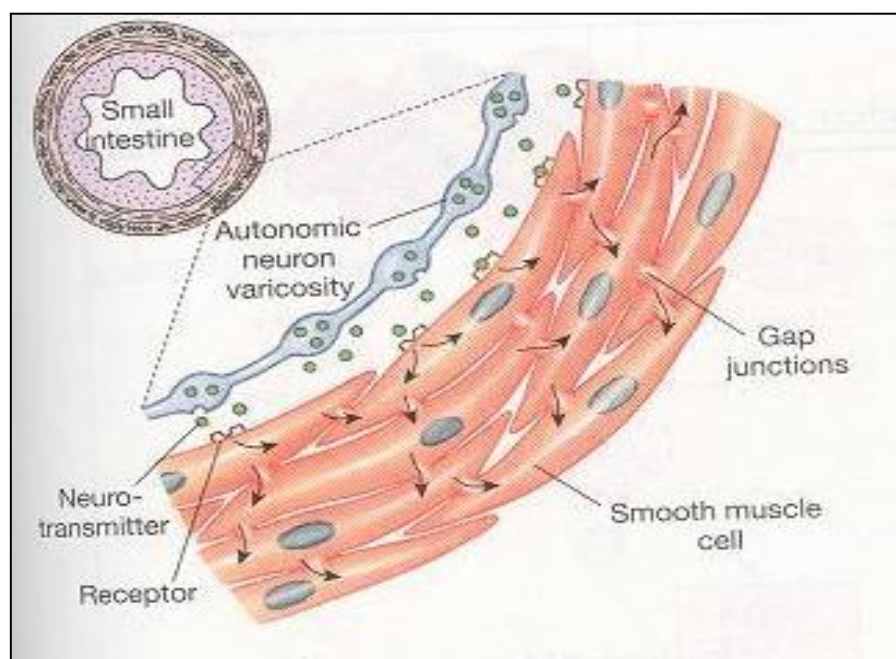
Smooth muscle is innervated by efferent fibres of the autonomic nervous system (both sympathetic and parasympathetic). It responds to both excitatory and inhibitory stimuli, which cause contraction and relaxation respectively (in contrast to skeletal muscle, which responds only to excitatory stimuli). Smooth muscle cannot be controlled voluntarily and is involved only with autonomic reflexes. The nerve fibers essentially pass "close" to the smooth muscle cells and release neurotransmitter (which is not restricted to acetylcholine) from swellings in the fibre, called varicosities. These are near to the surface of effector cells; smooth muscle has no specialised postsynaptic membrane.

Smooth muscle also responds to adrenaline (in bronchodilation, for example), angiotensin II, vasopressin, drugs, propranolol (betablocker) and stretch (peristalsis).



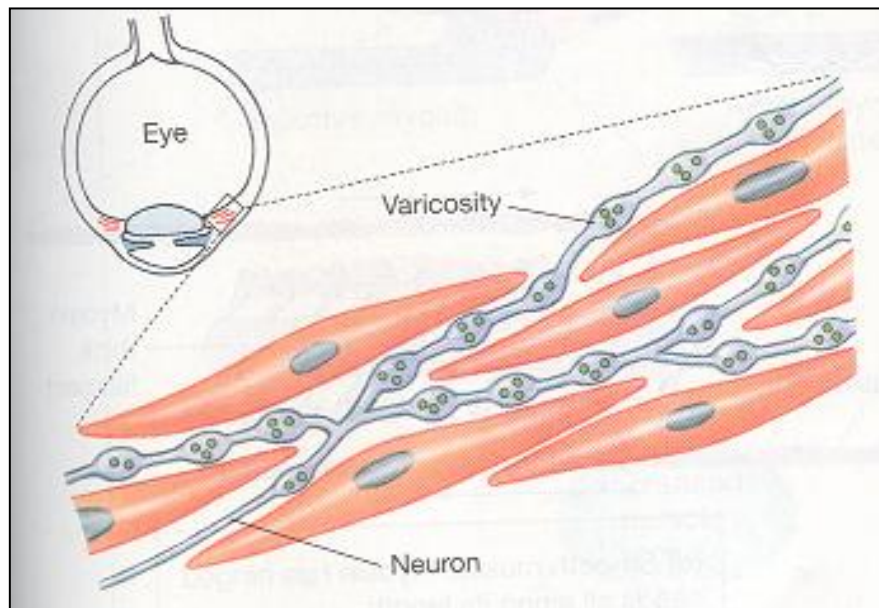
Single-Unit Smooth Muscle

In single-unit smooth muscle, individual smooth muscle cells are electrically coupled via gap junctions (also called nexi). Depolarisation spreads from one cell to the next.

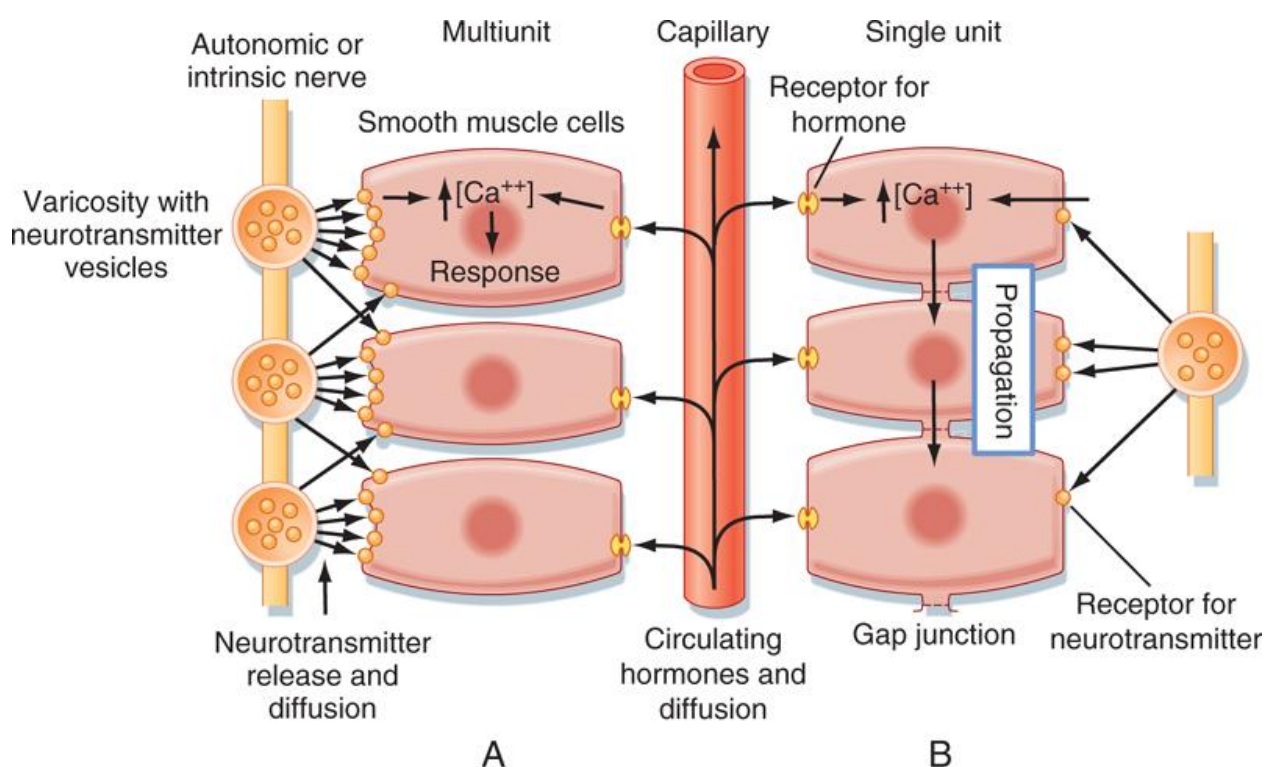


Multi-Unit Smooth Muscle

In multi-unit smooth muscle, each smooth muscle cell must be stimulated by an axon (meaning that the muscle is densely innervated). Few (or no) gap junctions are present.

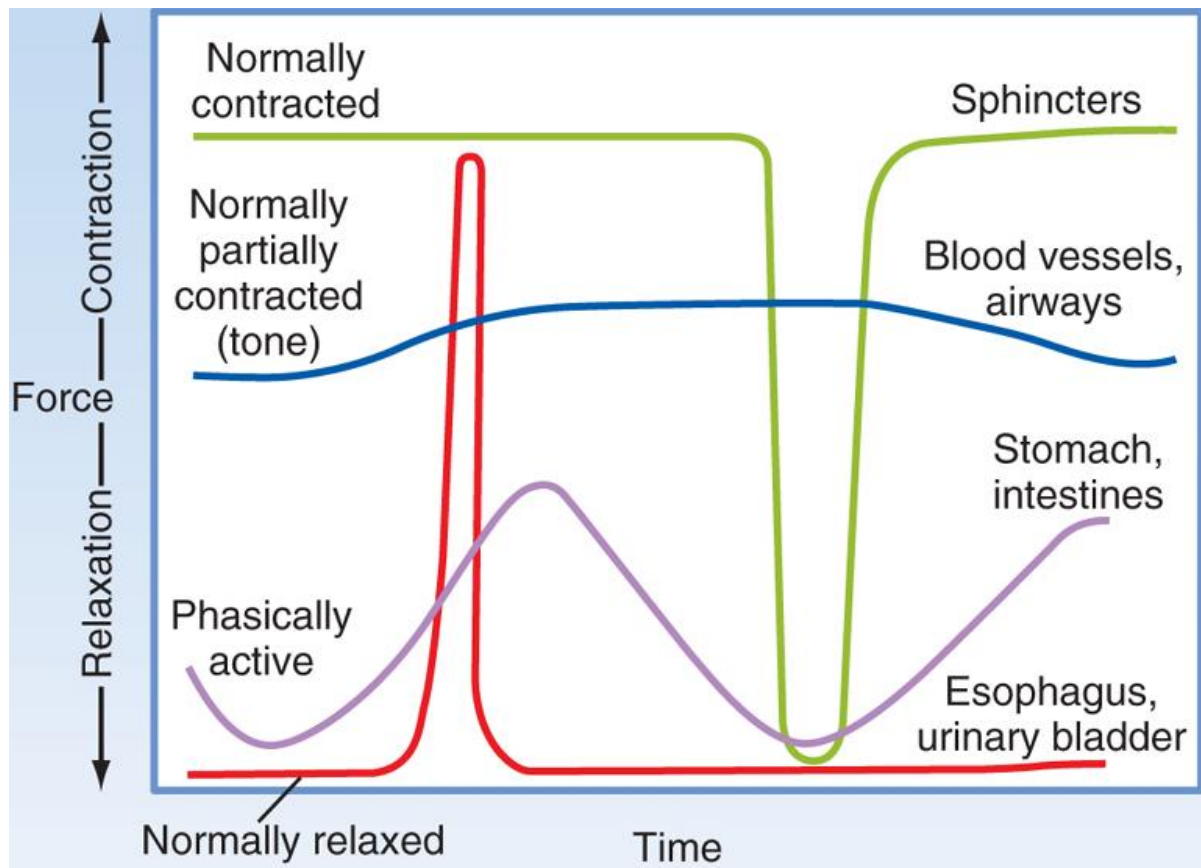


The two types are summed up in the diagram below.

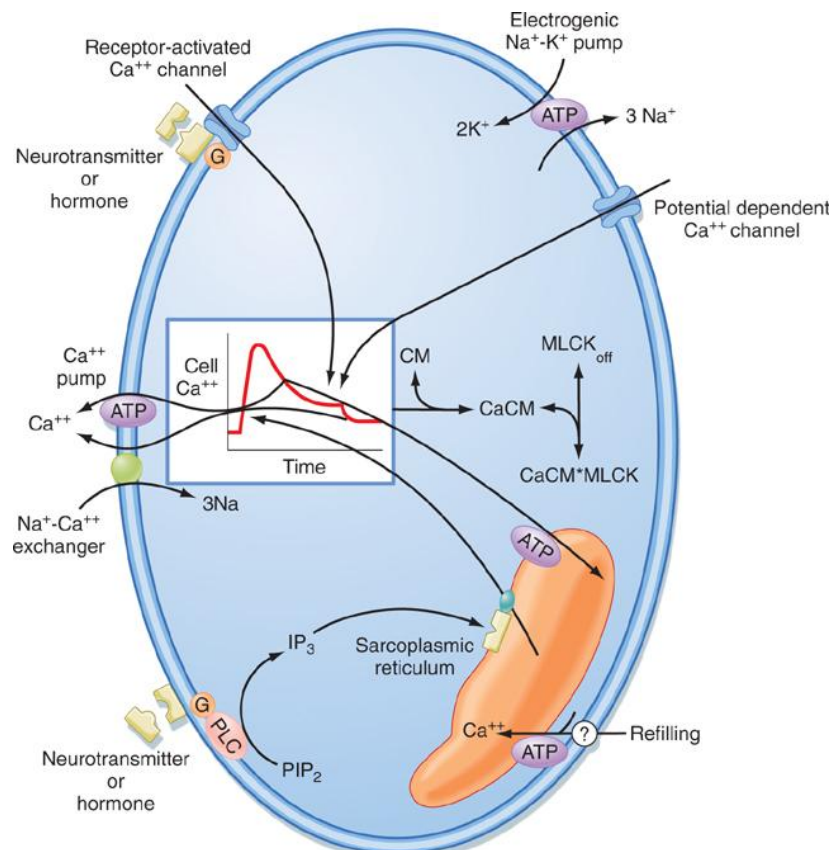


Koeppen and Stanton: Berne & Levy Physiology, 6th Edition.
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Smooth muscle has an unstable resting membrane potential. This results in “slow waves”, rhythmic contractions of smooth muscle. Pacemaker cells dictate the rate at which slow waves fire.



Sarcoplasmic Reticulum and Calcium in Smooth Muscle



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As with skeletal muscle, calcium is released from the SR. However, in smooth muscle, the SR is less well organised; it is adjacent to the membrane and its invaginations (caveoli). However, most of the calcium for coupling comes from the extracellular fluid, not the sarcoplasmic reticulum.

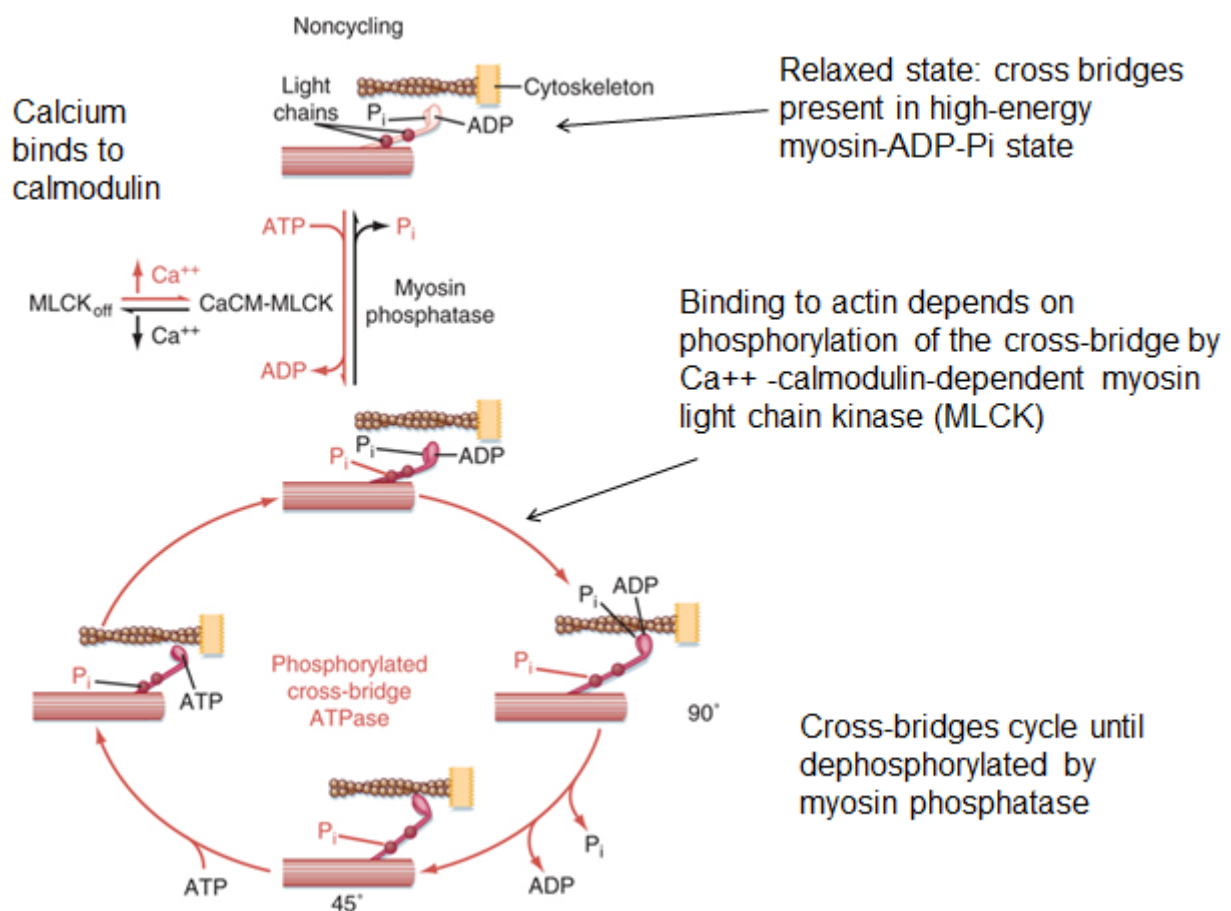
Calcium enters smooth muscle cells through their membranes during an action potential via voltage dependent calcium channels. Neurotransmitters can cause entry of calcium through receptor operated calcium channels as well as cause the release of calcium from intracellular stores (via second messengers such as IP_3).

Contractile Activity of Smooth Muscle

This is very similar to skeletal muscle and works via the sliding filament theory, involving crossbridge cycling, twitches, summation and tetanic contractions. It is activated by Ca^{2+} . Generally, it is much slower, sustained and very efficient in comparison to skeletal muscle.

Latch State of Smooth Muscle

The latch state of smooth muscle enables sustained smooth muscle tone with a low rate of cross-bridge cycling (and therefore a low rate of ATP usage). It occurs when some of the cross-bridges attached to thin filaments become dephosphorylated (by myosin phosphatase). This greatly slows the rate of cross-bridge detachment; filaments tend to remain “locked” together.



Muscle Relaxation

Neurotransmitters can, via second messengers, result in the phosphorylation of enzymes involved in pumping out calcium across the membrane. This enhances their activity and causes a decrease in calcium concentration, which results in relaxation.

Clinical Aspects

Pathological behaviour of smooth muscle may be involved in many illnesses such as hypertension. However, smooth muscle cells divide which means that they can regenerate after injury.

Summary and Comparison of Skeletal, Cardiac and Smooth Muscle Types

TABLE 10–4 A Comparison of Skeletal, Cardiac, and Smooth Muscle Tissues			
Property	Skeletal Muscle	Cardiac Muscle	Smooth Muscle
Fiber dimensions (diameter × length)	100 μm × up to 30 cm	10–20 μm × 50–100 μm	5–10 μm × 30–200 μm
Nuclei	Multiple, near sarcolemma	Generally single, centrally located	Single, centrally located
Filament organization	In sarcomeres along myofibrils	In sarcomeres along myofibrils	Scattered throughout sarcoplasm
SR	Terminal cisternae in triads at zones of overlap	SR tubules contact T tubules at Z lines	Dispersed throughout sarcoplasm, no T tubules
Control mechanism	Neural, at single neuromuscular junction	Automaticity (pacemaker cells)	Automaticity (pacesetter cells), neural or hormonal control
Ca²⁺ source	Release from SR	Extracellular fluid and release from SR	Extracellular fluid and release from SR
Contraction	Rapid onset; may be tetanized; rapid fatigue	Slower onset; cannot be tetanized; resistant to fatigue	Slow onset; may be tetanized; resistant to fatigue
Energy source	Aerobic metabolism at moderate levels of activity; glycolysis	Aerobic metabolism, usually lipid or carbohydrate substrates	Primarily aerobic metabolism (anaerobic during peak activity)

Structure of Tendons, Ligaments, Bone, Cartilage and Ossification

Tendons and Ligaments

Introduction

As a general rule, tendons attach muscles to bones (NB some simply link two muscle bellies together) and thus a tendon transmits the movement generated by a muscle belly to the skeleton. Ligaments attach bone to bone (usually two different bones, but occasionally different parts of the same bone) and guide and limit joint movements. A typical tendon extends from a myotendinous junction to an enthesis (i.e. the bone-tendon junction [osteotendinous junction]); a typical ligament extends from one enthesis (i.e. the bone-ligament junction) to another. Both are dense connective tissues dominated by regularly arranged collagen fibres and have high tensile strength.

Function of Tendons

In transmitting the pull generated by a muscle, a tendon is part of a continuum of connective tissue linking contractile cells with their targets. Thus, the connective tissues of a muscle blend into a tendon, which in turn attaches to a bone. The force transmission role is well understood; however it is less well appreciated that there are several other important aspects of tendon function. Tendons:

- Transfer force from muscle to bone to generate movement.
- Allow the muscle belly to be some distance from its site of action. This allows the muscle(s) to pull through a narrow space (e.g. carpal tunnel).
- Enable the pull of a muscle to be accurately focused onto single or multiple sites, or for several muscles to act on one site.
- Eliminate the need for an unnecessary length of muscle between origin and insertion, allowing the length of the muscle belly to be appropriate to the amount of movement required - the longer the muscle belly, the greater the range of movement.
- Change the direction of pull of a muscle by wrapping around a bony pulley.
- Reinforce or replace part of a joint capsule.
- Act as springs that store energy in locomotion.
- Hold other tendons in position - e.g. the superficial flexor tendons of the digits hold the deep ones in position.
- Prevent subluxation (dislocation).

Tendons are also prone to pathology – especially in athletes; both elite and recreational (overuse injuries).

Functions of Ligaments

Joint movements are guided and limited by ligaments that may be local thickenings of the capsule or separate accessory ligaments. The position, size and shape of ligaments relate to the forces acting on the joint - they must limit allowable movements and prohibit unwanted ones. As with tendons, there are other functions that are seldom appreciated. Ligaments:

- Attach bone-to-bone.
- Stabilise, limit and guide joint movement.
- Provide attachment for muscles (e.g. the interosseous membrane greatly increases the area available for the attachment of forearm muscles).
- Send signals to the brain that are important in proprioception and the active maintenance of joint stability.
- Are modified to form articular discs in synovial joints.
- May act as 'guy ropes' to tie down other soft tissues to bone. Thus as retinacula, they bind down tendons, preventing them from 'bowstringing'.

Specialised Regions

There are 3 specialised regions of tendons or ligaments - the *myotendinous junction* (restricted of course to tendons), the bone-tendon or bone-ligament junction (called the '*enthesis*' by rheumatologists) and the regions where some tendons change direction by wrapping around bony pulleys (*wrap-around regions*). Each of these is subject to particular diseases or injuries.

Myotendinous junctions: The myotendinous junction is where force is transferred from muscle to tendon and where new sarcomeres are added during muscle growth. The junctions show striking adaptations for force transfer e.g. extensive infoldings of the muscle cell membrane that significantly increase the surface area for tendon contact and reduce stress concentration. The contact area is dramatically reduced after limb immobilisation. This suggests that the tensile strength of the myotendinous junction is reduced by immobilisation and that this predisposes it to injury. Progressively increasing levels of exercise during the recovery period probably reduce the chances of re-injury. The myotendinous junction is where muscle strains and delayed onset muscle soreness commonly occur. Strains are typical of eccentric contractions, where muscles lengthen while producing a force greater than that in concentric contractions. Muscles crossing 2 joints are at particular risk and it is probably significant that their tightness limits joint movement, that they commonly act eccentrically in athletic activities and that they contain many fast twitch fibres. Strains are particularly characteristic of sports that involve fast running or rapid acceleration. Eccentric contractions are again the most likely trigger in delayed onset muscle soreness.

Enteses: Enteses are frequent sites for joint pathology and the conditions are collectively known as 'enthesopathies'. They include some common problems like tennis elbow, calcaneal bony spurs and DISH, but it is important for you to note that the enthesis is the prime target organ in a whole host of conditions collectively known as the seronegative spondyloarthropathies – the best known of which is ankylosing spondylitis. In severe cases, bone grows from one ligament enthesis to another in the spine, resulting in complete fusion or 'ankylosis' of some regions of the spine.

There are two fundamentally distinct forms of enthesis. Typically, those near the ends of long bones or on the short bones of the hand and foot, attach to bone via a region of fibrocartilage, whereas those attaching to the shafts of long bones are purely fibrous. Because ligaments are associated with joints and because most tendons attach just beyond the joint on which they principally act, fibrocartilaginous attachments are the most common. There is an important mechanical difference to appreciate between a tendon that attaches close to a joint and one that attaches far away. This can be illustrated by comparing the attachment of deltoid with that of supraspinatus. As the arm is moved away from the body, the angle between the tendon of supraspinatus and the humerus 'opens up' whereas the tendon of deltoid still approaches the arm at an acute angle. This means that there is an increased risk of wear and tear at bone-tendon junctions in tendons or ligaments that attach near the ends of long bones and this is why tendons like supraspinatus are fibrocartilaginous and ones like deltoid are fibrous.

The fibrocartilage probably controls the bending of the fibres and ensures that it does not occur at the hard tissue interface, but is displaced gradually through the fibrocartilage and into the tendon itself. It has a role rather like that of the flexible grommet where an electric cable joins a plug - the grommet ensures that the cable bending occurs away from the electrical connections. Enthesis fibrocartilage is just one of a series of protective devices to reduce wear and tear. At certain attachment sites such as the Achilles tendon, it is accompanied by:

1. A small synovial bursa that allows the tendon to move freely relative to the bone
2. A sesamoid fibrocartilage on the deep surface of the tendon and a periosteal fibrocartilage on the bone that protects the tendon/ligament where they rub against each other/.

These can frequently be the site of enthesopathies such as the retrocalcaneal bursitis of athletes. The enthesis fibrocartilage itself can develop bony spurs that grow into the tendon by endochondral ossification and cause pain.

In several regions of the body, notably the hand and foot, tendons can change direction one or more times before reaching their final destination. These are known as wrap around regions. Tendons do this by wrapping around bony pulleys such as the malleoli or passing beneath fibrous retinacula. Ligaments too can bend around bone, such as the annular ligament that holds the head of the radius in place or the transverse ligament of the atlas that presses against the posterior surface of the dens. One pulley alone does not increase the mechanical advantage of a tendon, but gives it a more favourable angle of approach. Some tendons pass around a whole succession of pulleys – (peroneus longus, for example).

When a tendon wraps around a pulley, it is subject to compressive and shear forces in addition to tensile ones. In order to withstand these forces, the composition of the tendon is specialised - it becomes fibrocartilaginous - because cartilage can withstand compression/shear. It seems that this fibrocartilage is a highly dynamic tissue. When wrap-around tendons are re-routed surgically so that the direction of the compressive forces is altered, the tendons modulate their structure in accordance with the new load. Wrap-around tendons are frequently subject to considerable wear and tear, notably fragmentation and delamination of the tendon surface.

There are a number of clinical conditions affecting tendons in the region of bony or fibrous pulleys. Again you should note that they are target sites in the seronegative spondyloarthropathies. In particular, tendons that wrap around the malleoli are commonly affected (for example, posterior tibial tendinitis). The overuse injury known as de Quervain's disease affects tendons of the thumb as they pass beneath the extensor retinaculum.

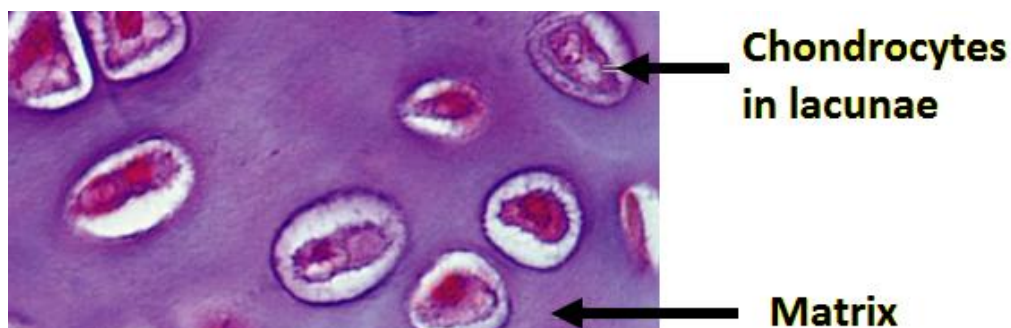
Cartilage and Bone

Cartilage

Cartilage is semi-rigid, strong, but slightly flexible. It withstands compressive forces such as weight-bearing, but can also be bent (such as in the expansion of the rib cage during breathing). Cartilage plays a major role during development and growth of long bones (endochondral ossification). It is avascular: cells in cartilage have to get their oxygen and nutrients by long range diffusion. The matrix contains chondrocytes in the small matrix-enclosed compartments termed lacunae. These started off as chondroblasts: they secrete matrix and become embedded within it (at which point they are called chondrocytes). Young chondrocytes still able to divide produce little clusters or cell nests within the matrix. There are three types of cartilage: hyaline, elastic and fibrocartilage.

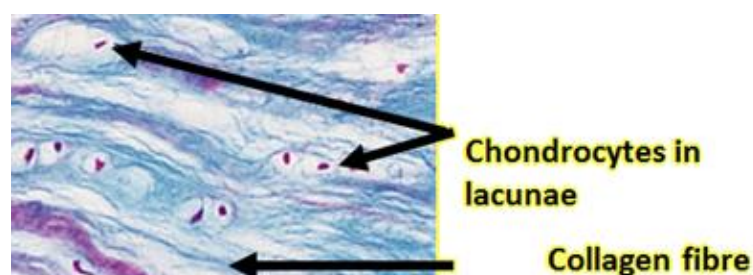
Hyaline Cartilage

Hyaline (pearly white and glassy) cartilage is found in the embryonic skeleton, the articular ends of long bones, nose and trachea; and forms the costal cartilages and larynx. The matrix in this cartilage appears pale blue in H&E sections, and is an amorphous gel composed of GAGs, proteins and glycoproteins with abundant fine collagen (type II) fibrils within it. Hyaline cartilage has a unique composition: it is strong enough to bear weight, but has a high content (75% of wet weight) of tissue fluid (mostly water) held in place by the gel structure of the matrix. This enables the oxygen and nutrients to diffuse from capillaries outside the cartilage itself to the innermost chondrocytes within their lacunae.



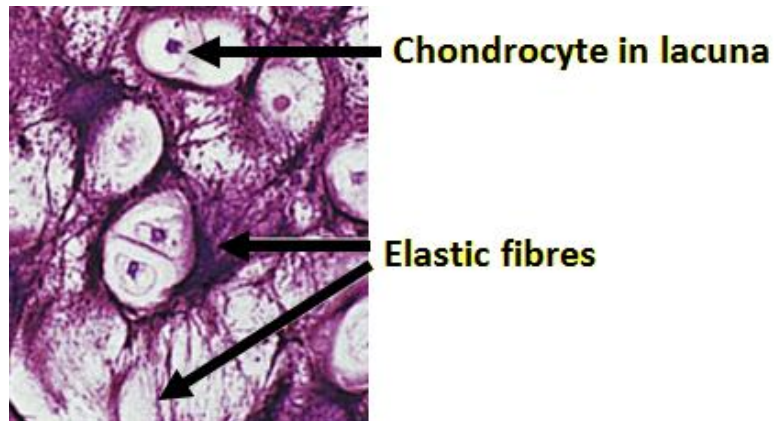
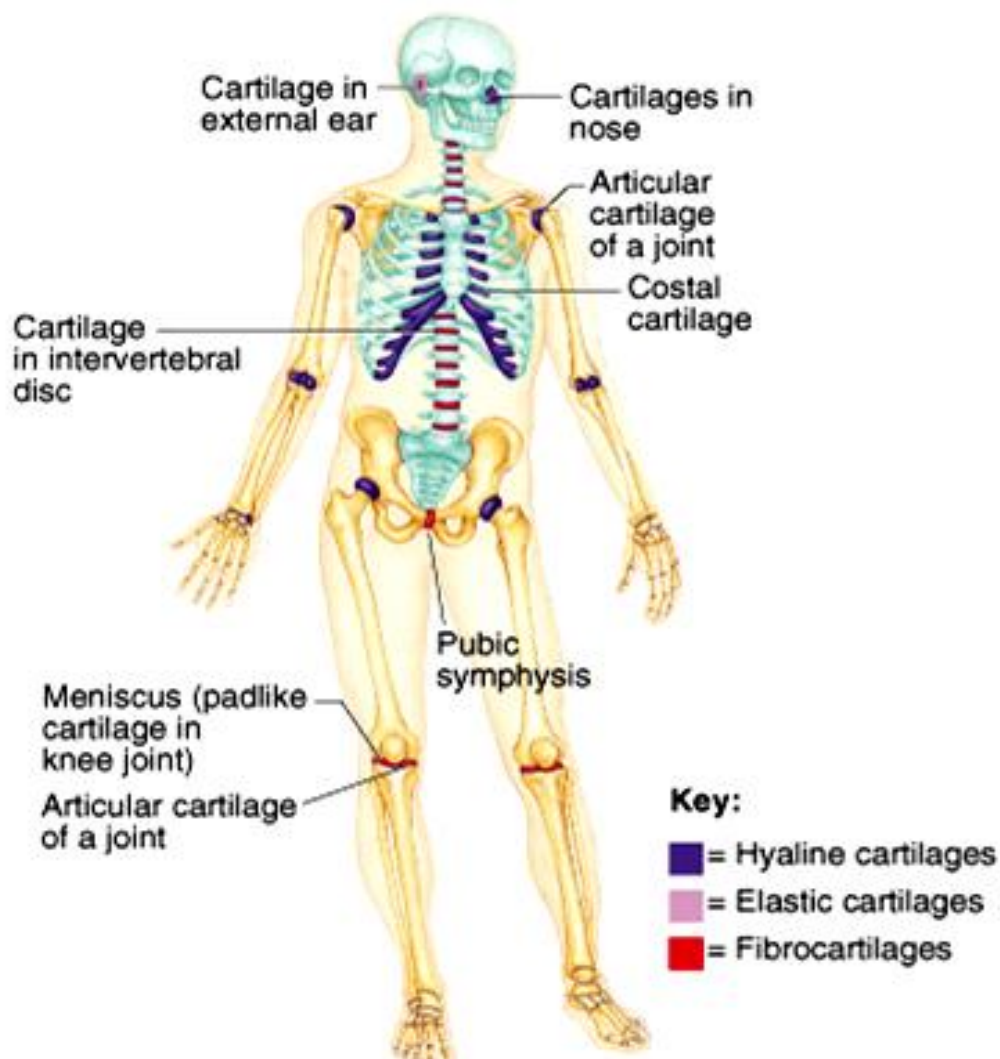
Fibrocartilage

Fibrocartilage is made up of prominent bundles of collagen fibres arranged in parallel to resist stretch. The chondrocytes are found in straight rows and there is no perichondrium (outer fibrous connective tissue sheath). Fibrocartilage is found at tendon insertions, in articular menisci, the pubic symphysis and in the intervertebral discs of the vertebral column. It provides tensile strength and absorbs compression shock.



Elastic Cartilage

Elastic cartilage is adapted to withstand repeated bending, but maintains its shape. It is found supporting the larynx, epiglottis and external ear (pinna). It is flexible, but able to spring back into its original position. It resembles hyaline cartilage, but in addition to widely dispersed collagen fibrils, the matrix also contains elastic fibres.

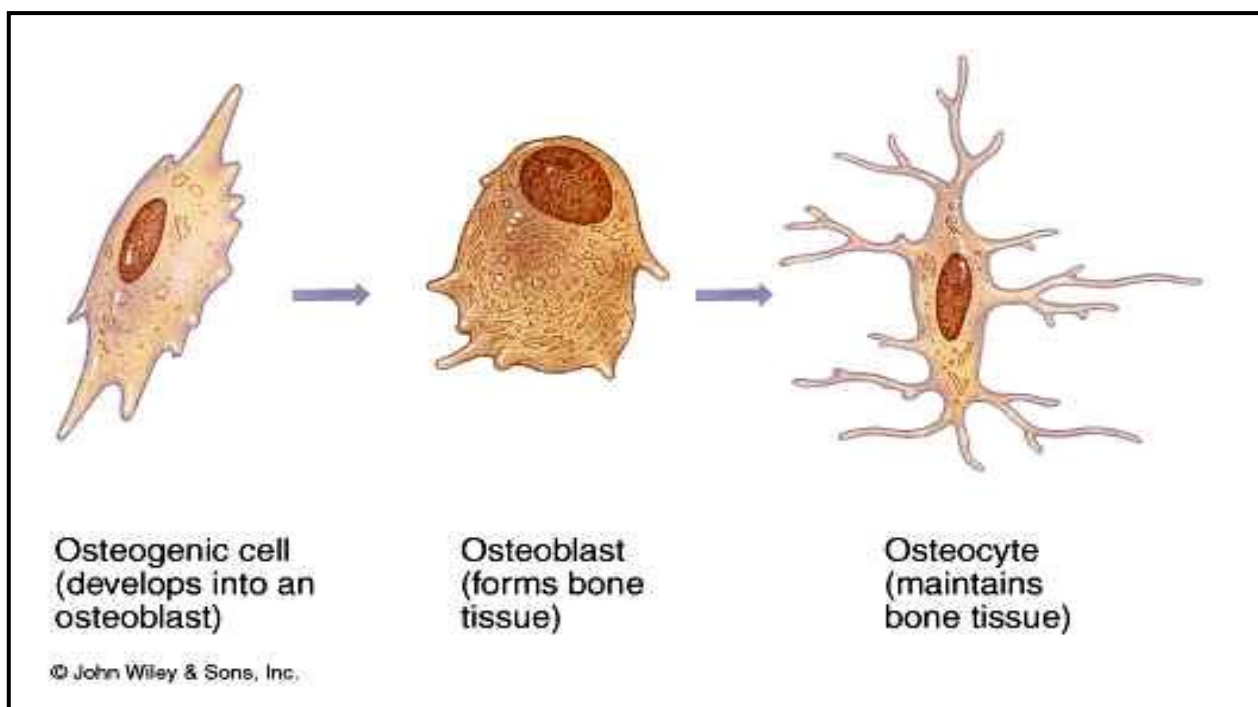
**Cartilage Locations in the Adult Skeleton**

Bone

Bone is a rigid form of connective tissue; it functions to support, protect and act as a storehouse for minerals. The marrow inside bones acts as a site for haemopoiesis. Collagen fibres represent 95% of the organic matrix. The fibres are embedded in glycosaminoglycans (GAGs) and mineral salts. The salts are mainly a crystalline form of calcium phosphate called hydroxyapatite.

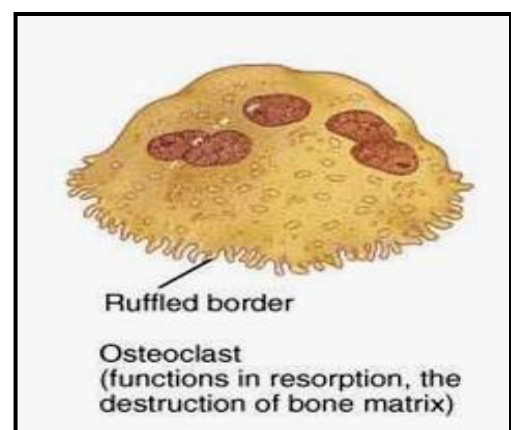
Bone Cells

Principal bone cells are osteocytes, and are found in small spaces in the mineralised matrix called lacunae. Small canals termed canaliculi connect adjacent lacunae. Within the canaliculi, there are cytoplasmic processes of the osteocytes, and these canals permit tissue fluid to flow from one lacuna to another, thus bathing the osteocytes. Osteoblasts are bone forming cells; osteoclasts are bone resorbing cells.

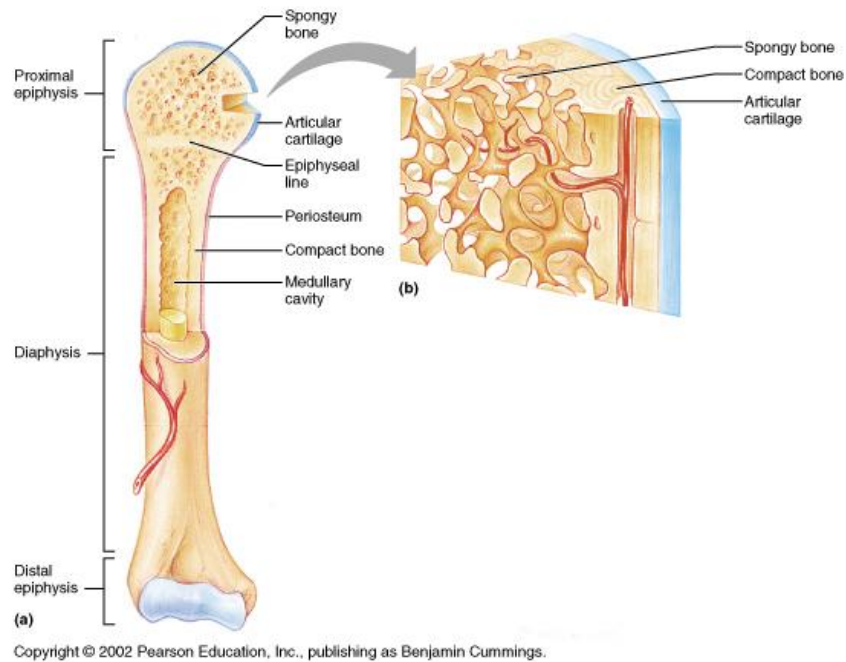


Osteoclasts

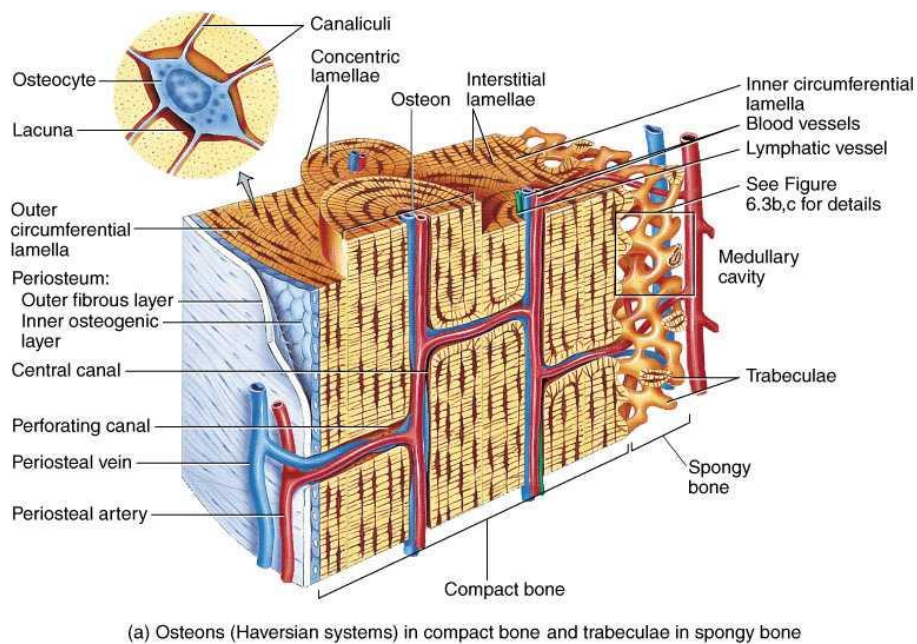
Osteoclasts are multinucleated cells; they function in resorption, the destruction of bone matrix.



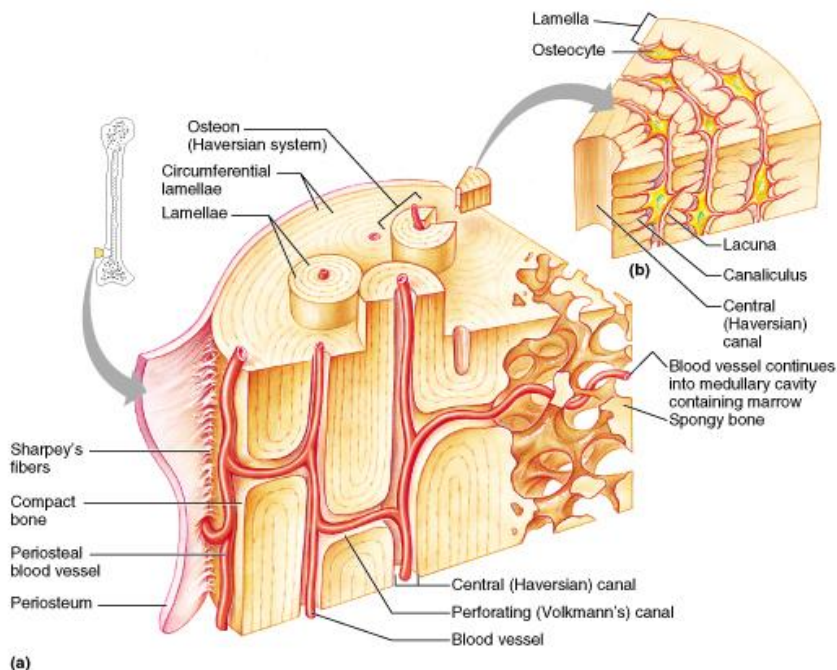
Two Forms of Bone: Compact and Spongy (Cancellous)



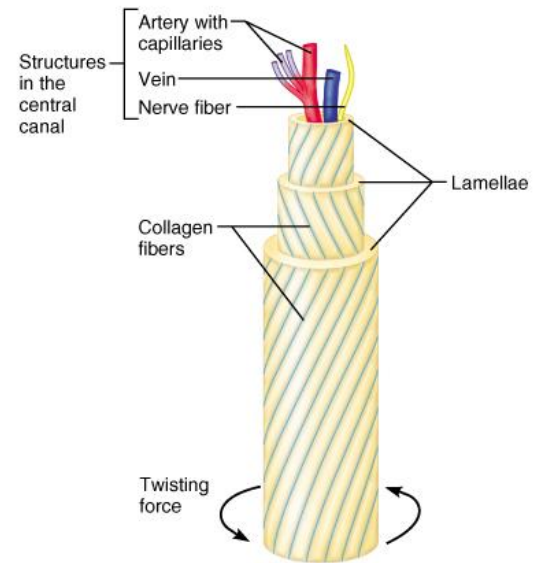
Compact Bone



Compact bone exists in osteons (or haversian systems).



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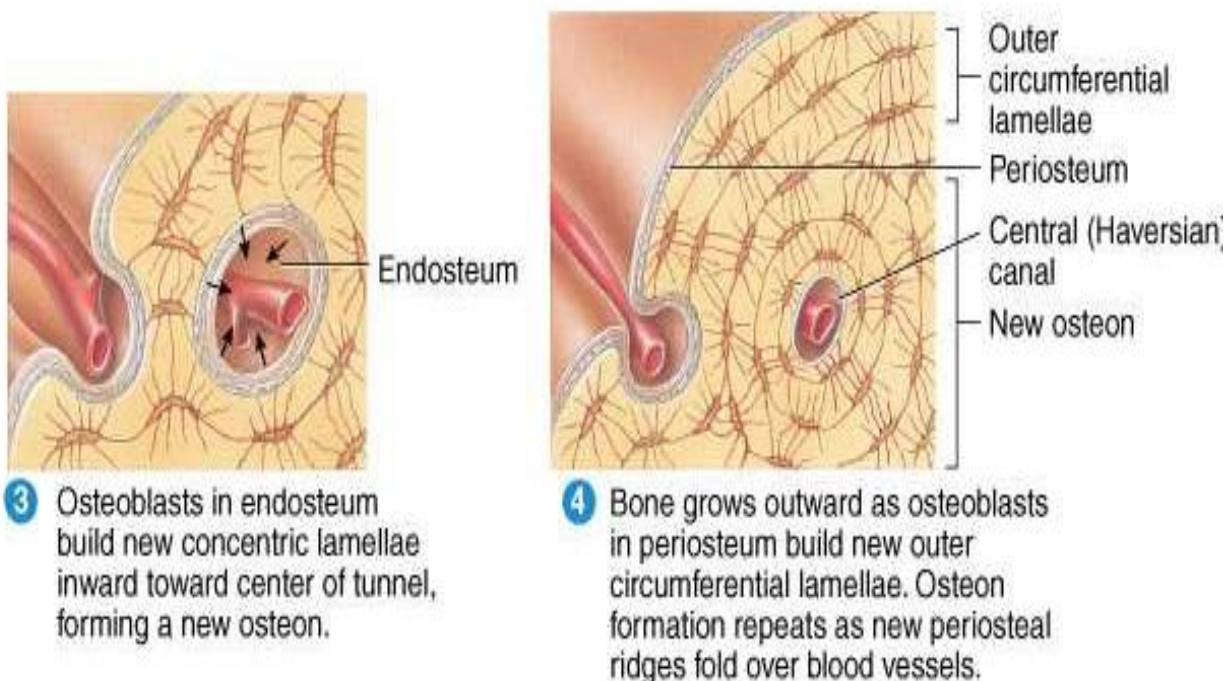


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Spongy Bone

Spongy bone does not contain osteons, but has trabeculae surrounding red marrow spaces.

Bone Growth in Osteons

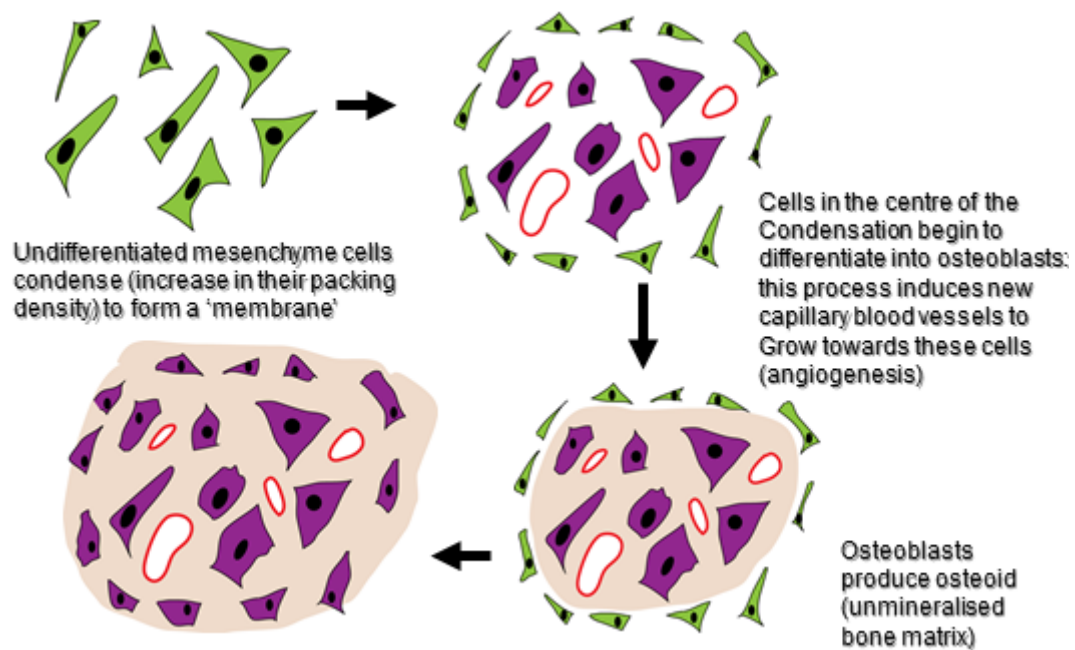


Bone Marrow

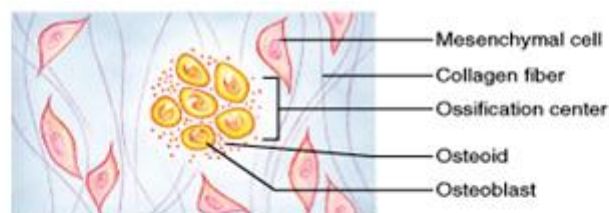
Bone marrow contains adipocytes, stromal cells and haemopoietic cells.

Ossification

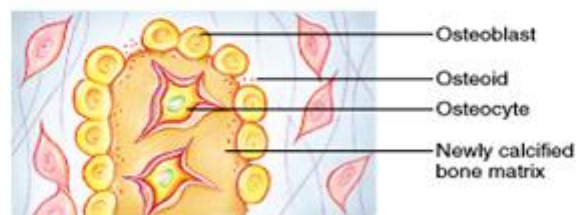
Intramembranous Bone Formation (For Example, Parietal Bone)



Intramembranous Ossification



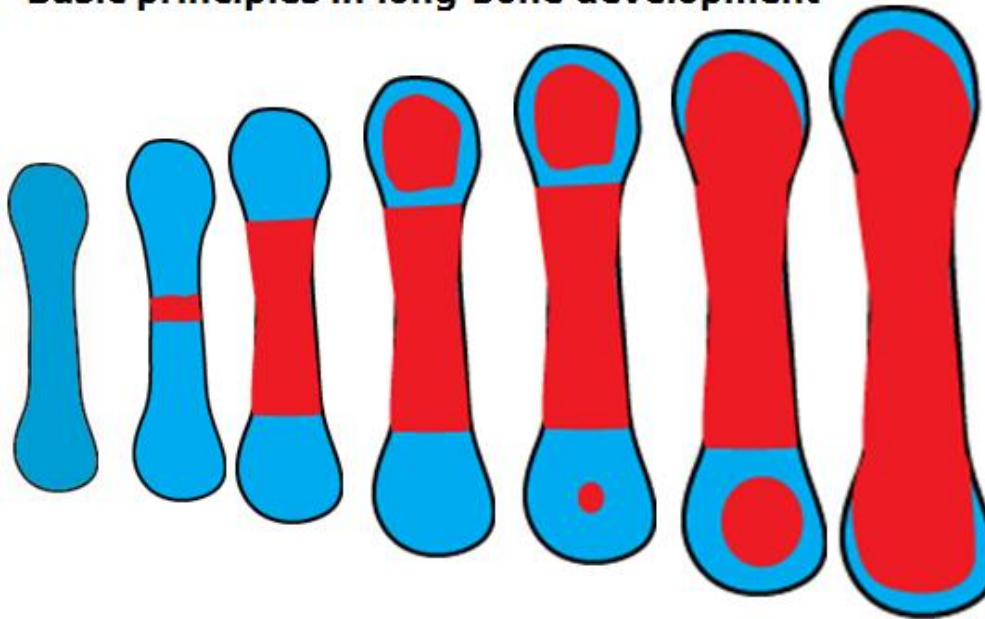
Ossification centre in condensed mesenchyme, central cells differentiate into osteoblasts



Bone matrix (osteoid) is secreted by the new osteoblasts- this becomes mineralised in a few days. The trapped osteoblasts become osteocytes

Endochondral Ossification

Basic principles in long bone development



The first image represents a hyaline cartilage model. The central cartilage calcifies and a periosteal bone collar forms in diaphysis (second image across). This expands through the hyaline cartilage which disintegrates; cavities start to appear, into which blood vessels invade. Bone is then deposited on calcified cartilage remnants.

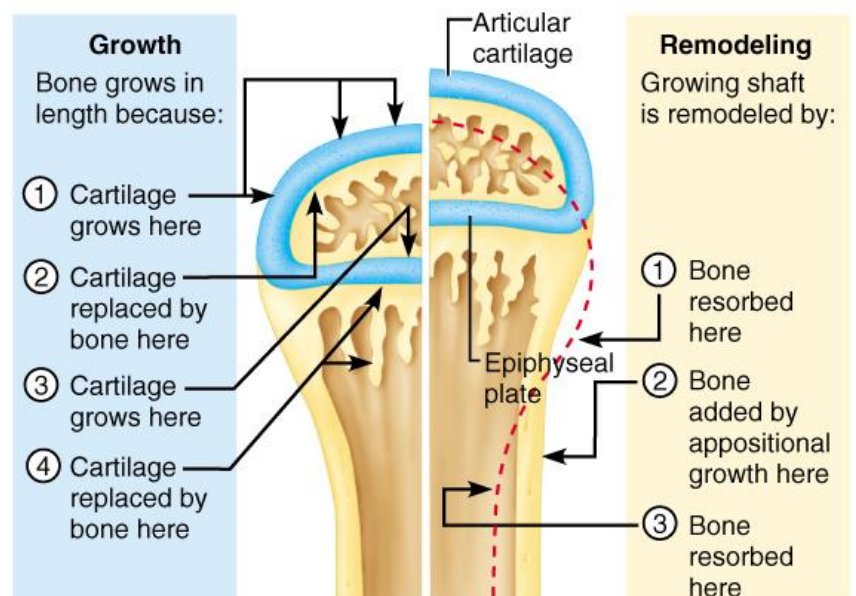
Hyaline cartilage remains at the epiphyseal surfaces (articular surfaces of joints); and at the epiphyseal growth plates between diaphysis and epiphysis (primary and secondary ossification centres on either side). The growth plate then closes at one end.

Ossification Timetable

Ossification begins at the 3rd prenatal month. Between birth and 5 years of age, secondary ossification centres appear. The bones of the upper limbs are completely ossified by 15-18 years in females and 17-20 years in males. By the age of 23 in females or 25 in males, nearly all bones are ossified.

Bone Remodelling

Bone deposition by osteoblasts occurs where bone is injured or needs extra strength. This requires vitamins C, A and D; calcium; magnesium; and the enzyme alkaline phosphatase. Bone resorption occurs through osteoclasts. The control of bone remodelling is a hormonal mechanism.



Connective Tissue Biology (Dr F Savage & Prof B Caterson)

Connective Tissue Biology 1: Collagen

Overview

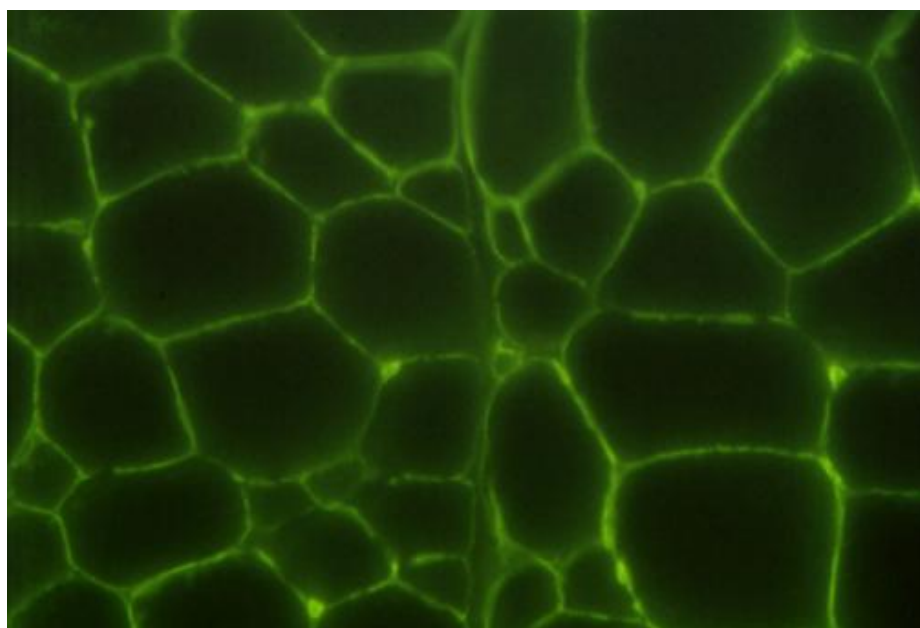
Connective tissue constitutes of mainly extracellular matrix, of which collagen is the principal constituent. Connective tissue has few cells; the main one is the fibroblast. The function of connective tissue is in contributing to the shape of an organ, resisting mechanical stresses and for support.

There are many diverse structures of connective tissue:

- | | |
|-----------------------|--|
| • Rope-like | tendons and ligaments |
| • Protective covering | skin |
| • Weight bearing | cartilage and intervertebral disc |
| • Skeletal | bone (calcification makes connective tissue very strong) |
| • Tubular | blood vessels and intestine |
| • Transparent | cornea |
| • Gels | vitreous humour |
| • Membranous | glomerular basement membrane |

Extracellular Matrix: Collagen

The extracellular matrix has several components. This lecture focuses on just one: collagen. Collagen is strong, provides tensile strength (acts as a scaffold) and reduces stretch. It affects cell behaviour by influencing polarity, proliferation, differentiation, migration and adhesion. The image below shows the extracellular matrix (green) surrounding individual muscle cells.



Collagen Overview

25% of body protein is collagen; it is the main protein of extracellular matrix (ecm) and is the most significant contributor to the strength of connective tissue. In conjunction with other ecm components it can influence the behaviour of cells. Collagen provides scaffolding for every organ through its contribution to the connective tissue and it forms a large percentage of demineralised bone and skin. Collagen is synthesised by mesenchymal cells, usually fibroblasts or the specialised equivalent in some tissue e.g. osteoblast in bone. It interacts with cells and other matrix components. There are many different types of collagen known, which differ in structure and hence in function and location.

Collagen Diseases

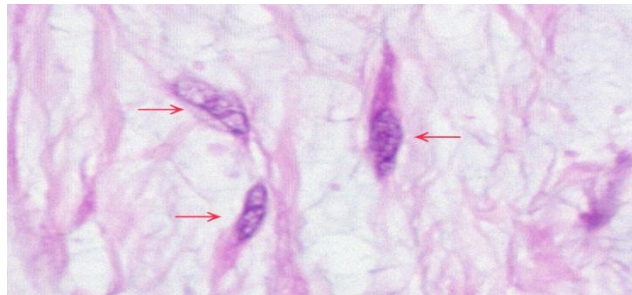
Specific collagen diseases are covered in the second lecture in the connective tissue series.

Degradation of collagen is a major part of the pathology but is secondary to the cause.

Degradation of collagen can occur in arthritis, osteoporosis, and invasion and metastasis of cancer.

Physiologically, collagen degradation (and synthesis) is important for growth and wound healing.

Collagen: Synthesis



Most connective tissues are derived from differentiated mesenchymal precursor cells (shown above). Mesenchymal precursor cells differentiate into fibroblasts in skin and tendons. What they differentiate into is different in certain locations. Examples are as follows:

- Osteoblasts in bone
- Chondroblasts and chondrocytes in cartilage
- Adipocytes in adipose tissue
- Myoblasts in muscle

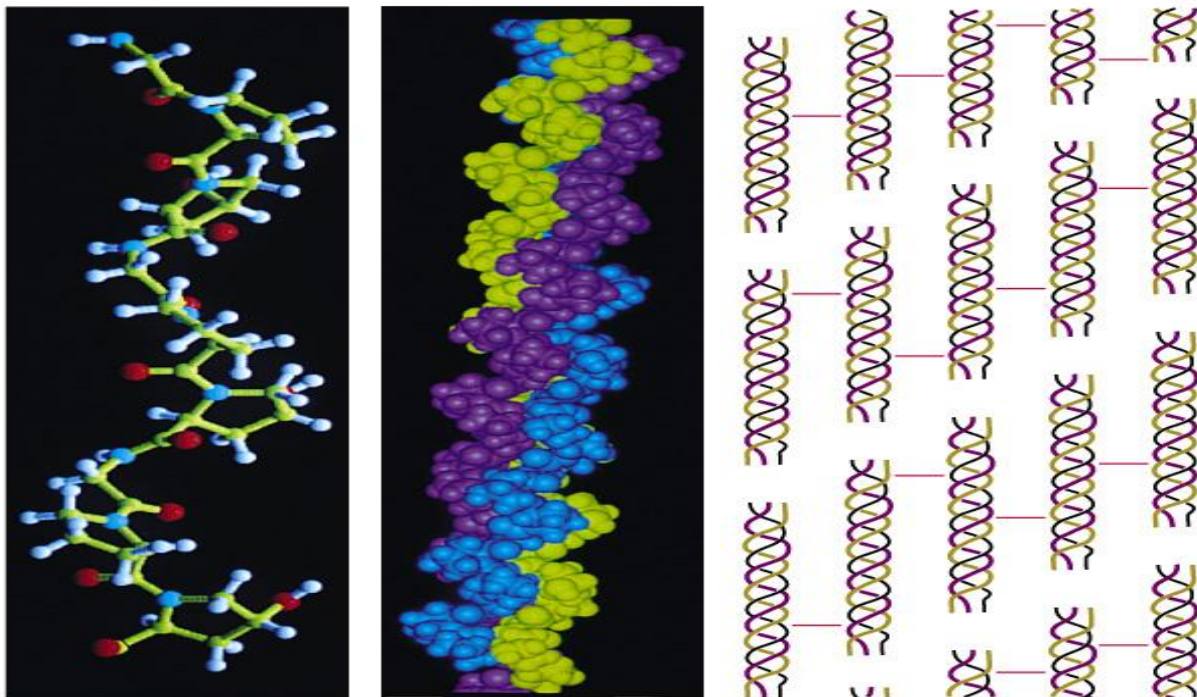
Collagens: Classification

There are six families of collagens and they are as follows (included are the families you need to know for each):

- Fibrillar collagens (I, II)
- Network collagens (IV)
- Folamentous collagens (VII)
- FACIT (IX)
- Membrane Bound
- Multiplexins

Collagen Structure: Multimeric Protein

Collagen has a long, stiff structure. It is very stable. For example, bone collagen has a very long half-life. Collagen forms a unique structure, the triple helix, which is formed of 3 peptide chains. The triple helix molecules join together to form fibrils and these unite to form bundles (fibres) that can be seen in tissues by light microscopy. These triple-helical regions are resistant to degradation and are not degraded by ordinary proteases. All collagens contain the triple-helical structure, however for some classes of collagen there are non-helical domains too. If the molecule is predominately formed of triple-helical collagen, with non-collagenous domains restricted to the C- and N- termini then the collagen molecules (triple helices) can aggregate to form fibrils. Fibrils do not form if helical regions are interspersed with non-helical regions. Collagen is rich in glycine and proline and also contains some unusual amino acids. Shown below from left to right are a peptide chain, a 3 chain form triple-helix and fibrils formed from triple-helices.



Collagen Structure: Peptide Chain

The Collagen Peptide has the repeating triplet amino acid sequence Gly-X-Y, in which X is often proline (very big) and Y hydroxyproline (also big). Glycine as every third residue is important because this is the smallest amino acid (its R group is hydrogen) and is the only one that can fit in the centre of the triple helix to allow tight packing of the helix. All glycine are on the same side of the chain. This allows packing to a superhelix because hydrogen can fit in its crowded centre. Proline and hydroxyproline have ring structures that help stabilise the molecule. Hydroxyproline and hydroxylysine are unusual amino acids found almost exclusive to collagen. They are formed by hydroxylation of proline and lysine during post-translational processing. Lysine and hydroxylysine allow cross-links to be formed between the collagen molecules (triple helices) to hold them together and if the cross-links cannot form the fibrils will be weakened.

Collagen Structure: Unusual Amino Acids

4 and 3-hydroxyl-L-proline are present in quantities of 10%, 0.5% respectively, though you should remember that they both represent about 10% of the amino acids present. These are not present in genetic code so are formed by post-translational modification of proline and lysine. These amino acids are rarely found in other animal proteins and therefore, hydroxyproline, which is more abundant than hydroxylysine, can be used as a measure of collagen. They are important as they form interchain hydrogen bonds that help stabilise the triple-helix.

To produce hydroxylate proline, ascorbic acid (Vitamin C) is required as a co-factor for the enzyme involved in the process and if this is absent then a disease called scurvy results. This is because the pro- α -chains are defective and can't produce a stable triple helix and immediately degrade within the cell. Therefore those areas that tend to have a slightly faster turnover of collagen than others become very fragile. Scurvy is seen most notably in blood vessels becoming very fragile and teeth becoming loose in their sockets. Scurvy was common in sailors who tended to be away for months without fresh fruit and veg and therefore had a deficiency in vitamin C.

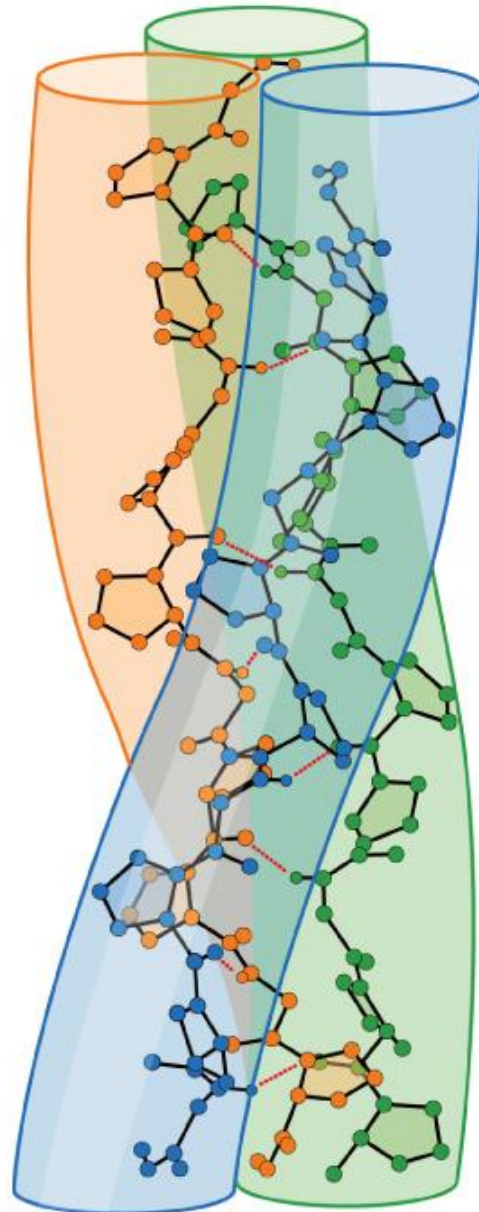
Collagen Structure: Triple Helix

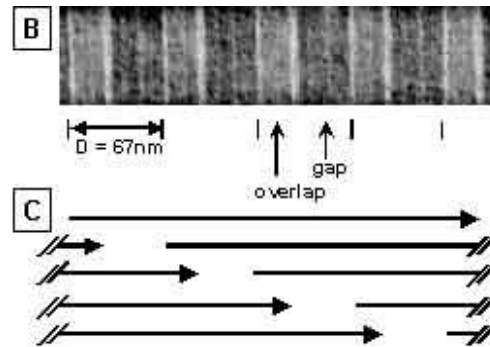
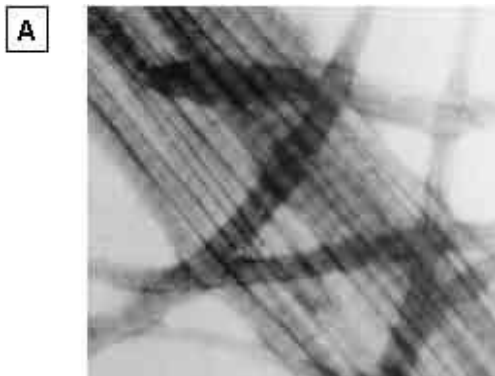
The triple helix is formed of 3 α polypeptide chains that can either be all the same, all different or 2 identical and 1 different. Different chains are coded by different genes and so knowing if a collagen type is homotrimeric (3 identical chains) or heterotrimeric has implications when considering the effect of a mutation in one of the collagen genes. The helix is right handed and has a unique structure.

Collagen Structure: Fibril Formation

The collagen molecules formed line up parallel with other molecules to form fibrils. This is not done in a random fashion but has a definite pattern.

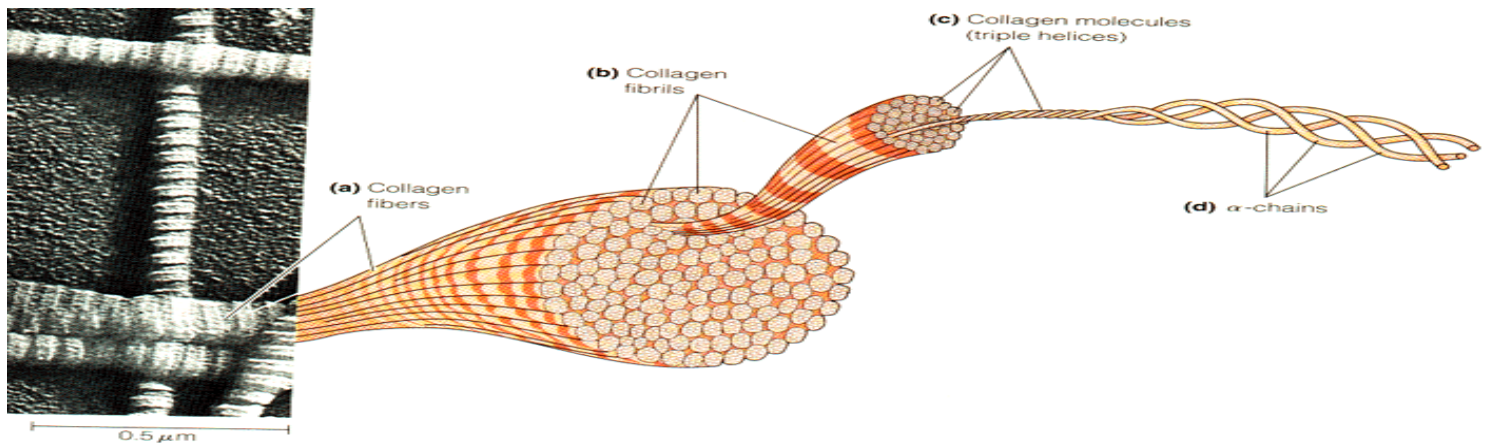
They don't lie exactly parallel to each other but in a staggered fashion, which leads to the characteristic banding pattern because there are areas in which many molecules are lined up and overlap zones and areas in which few collagen molecules are aligned, so an 'apparent hole' is formed. The cross-striations are around 67nm long.



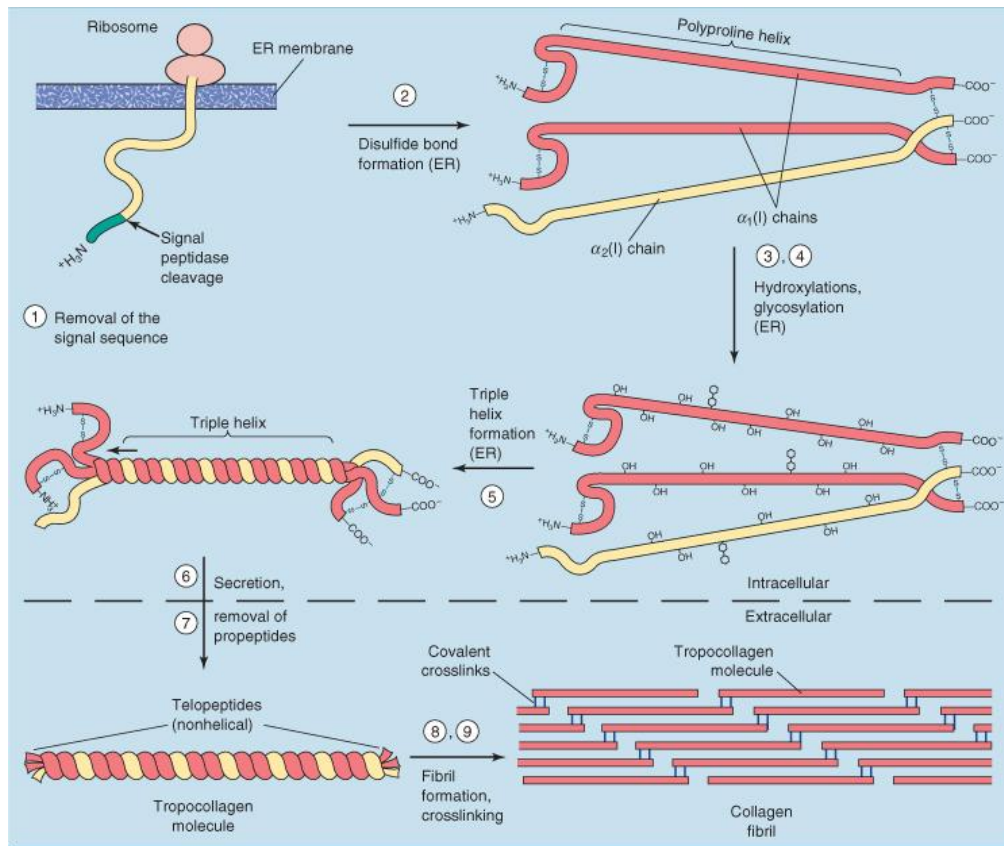


Collagen Structure: Fibres

After the triple helices align laterally to form fibrils, the fibrils can come together to form collagen bundles. These can be several millimetres in diameter. The arrangement of the bundles depends on the function of the tissue. For example, in a tendon, the bundles align in parallel as the forces are unidirectional; whereas in skin the arrangement is multi-directional to be able to resist forces in all directions. In bone and the cornea there are layers of collagen arranged parallel but at right angles to each other. A 1mm fibre carries 10kg of weight.



Collagen Synthesis: Overview



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Collagen Synthesis: Steps

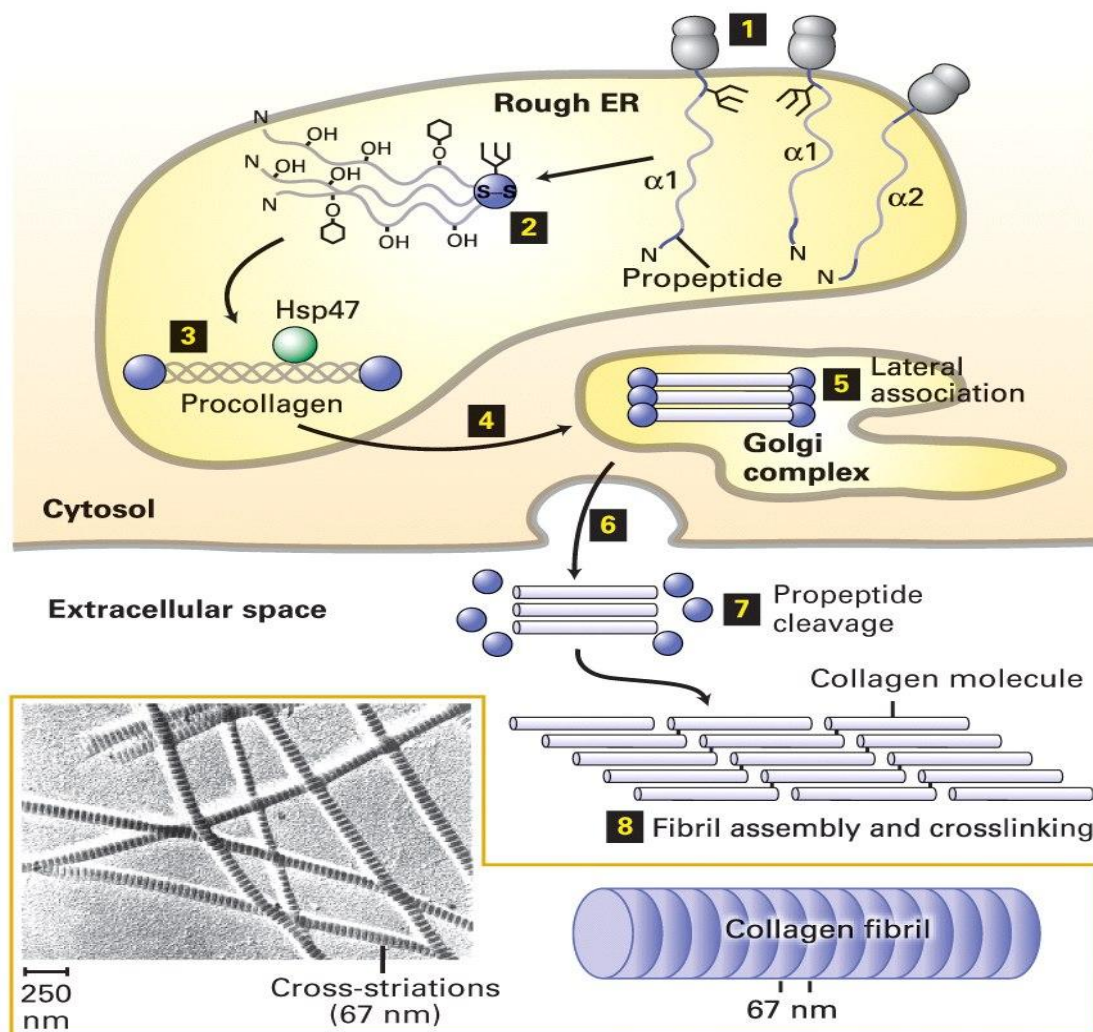
Intracellular processes:

- Transcription
- Translation
- Proline and lysine hydroxylation
- Glycosylation of hydroxylysine
- Triple helix formation
- Secretion

Apart from transcription and translation themselves, all of the other processes are post translational modifications.

Extracellular Processes

- Propetide removal
- Fibrillogenesis
- Crosslink formation



Collagen Synthesis: Processing of Peptides

Initially, there are non-helical regions at the N- and C- terminals. The N-terminal signal peptide is removed after ER. The C and/or N-terminal may contain many oligosaccharide units. Disulphide bonds form at the terminals only: intrachains at the N-terminal and interchains at the C-terminal.

Collagen Synthesis: Intracellular Modifications

Hydroxylation of proline and lysine requires enzymes, notably prolyl hydroxylases or lysyl hydroxylases. It also requires co-factors, specifically Fe^{2+} , O_2 , ascorbate (vitamin C) and 2-oxoglutarate.

Glycosylation of procollagen chains is the addition of galactose and glucosyl-galactose to some hydroxylysine. This is dependent on collagen type, location and age.

Collagen Synthesis: Helix Formation and Secretion

This involves a complex helical folding which is initiated by three C-terminal propeptides. Three chains come together and fold COOH to the NH_2 direction. A stable triple-helix formation requires glycine to be every third amino acid, and a high percentage of Y to be hydroxyproline (in the canonical collagen structure of X-Y-Gly). The propeptide is secreted and incorrectly coiled molecules are degraded intracellularly.

Collagen Synthesis: Extracellular Processing and Fibril Formation

This process is not well understood, but involves an aggregation self-assembly process. There is specific cleavage of N- and C- terminal propeptides by enzymes, which allow aggregation to fibrils.

Collagen Structure: Cross-Linking

Cross-linking provides tensile strength and mechanical stability. The covalent bonds between collagen molecules are formed between specific lysyl and hydroxylysyl residues. The process is initiated by the enzyme lysyl oxidase. This recognises a quarter stagger arrangement and requires copper and oxygen to function. Cross-links are essential for a strong fibre.

Examples of Different Classes of Collagen

Not all classes of collagen are “pure” triple helix. Some may have triple helical regions interspersed with non-helical domains (also called non-collagenous domains). Fibrils only form if the collagen is predominantly triple helix.

Fibrillar Collagens: e.g. Type I

These are predominantly triple helix with small non-collagenous domains at their N- and C-termini. These rod-like molecules are 300nm in length and their main function is mechanical strength. Types I, II and III form 80-90% of collagen in the body, with type I being the main type.

Each chain of type I collagen is 300nm long, consists of 1050 amino acids and has a diameter of 1.5nm. They are arranged into tightly packed, thick bundles and have an enormous tensile strength. Gram for gram, type I collagen is stronger than steel. It can be associated with FACIT (Fibril Associated Collagens with Interrupted Triple Helices) and filamentous collagens, and is a major collagen in skin, tendon, bone and the cornea.

Fibrillar Collagens: Tissue Organisation

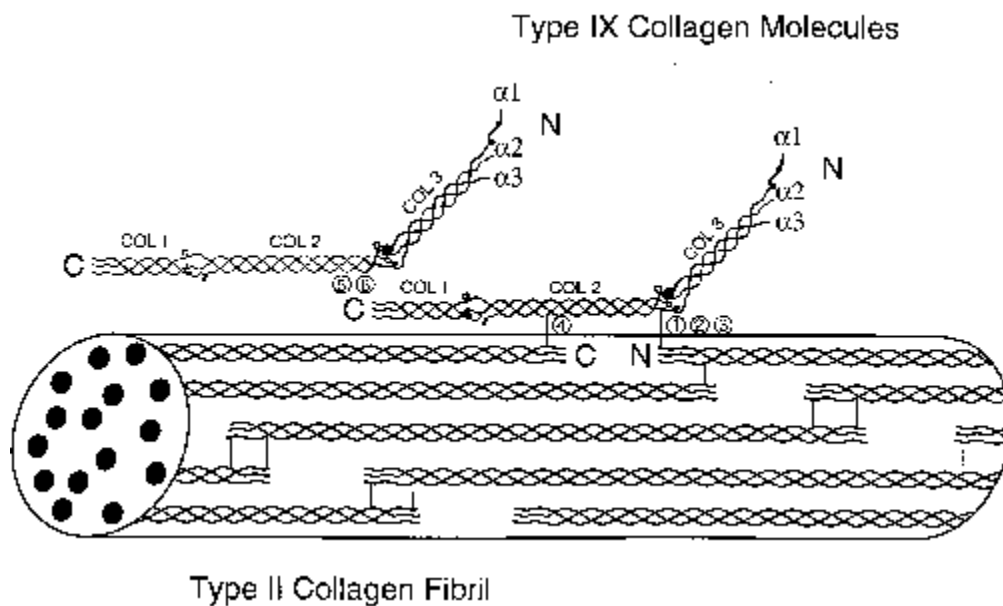
In the skin, collagen is organised in a wickerwork pattern which is multi-directional. It resists tensile strength in all directions. In tendons, collagen is aligned along the major axis of tension, in parallel. In bone and cornea, collagen is arranged in orderly layers. Within each layer, each fibril is parallel, but at right angles to adjacent fibrils.

Fibril-Associated Collagens (FACIT), e.g. Type IX

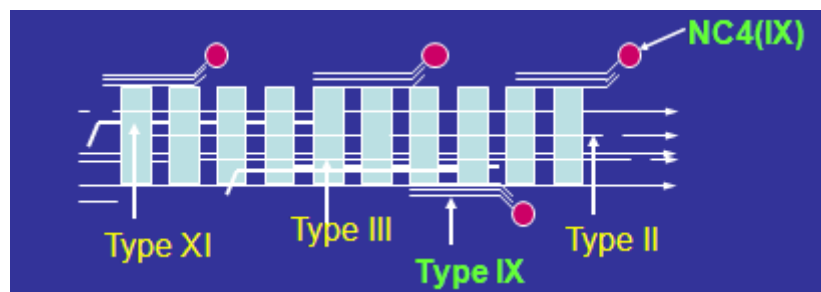
These interact with fibrillar collagens which affect their organisation. They contain non-helical regions so are more flexible than fibrillar collagens and are never found alone. They contain non-helical domains and retain pro-peptides, and therefore cannot form fibrils. They bind to the surface of other collagens. For example, Type VI and XII bind to type I collagen, and type IX binds to type II collagen.

Articular Cartilage: Interactions of FACIT and Fibrillar Collagens

In cartilage, type II is the main fibrillar collagen type (85-90%) and provides mechanical strength. 1-3% of collagen is type IX (FACIT). Below, a diagram shows their arrangement.



Interaction of Multiple Collagen Types



This example shows the presence of multiple collagen type forming a fibril. It is mainly composed of type II, with subsidiary types being III, IX and XI.

FACIT: Functions of Type IX Collagen

Type IX collagen regulates type II collagen fibril diameters by influencing lateral association of fibrils. Its location on the surface of fibrils is ideally situated to participate in interactions with other matrix components. Type II collagen is important for strength whilst the minor collagens are important for organisation. Loss of type IX collagen may lead to a defective cartilage matrix and diseases such as osteoarthritis.

Network Collagens: e.g. Collagen Type IV

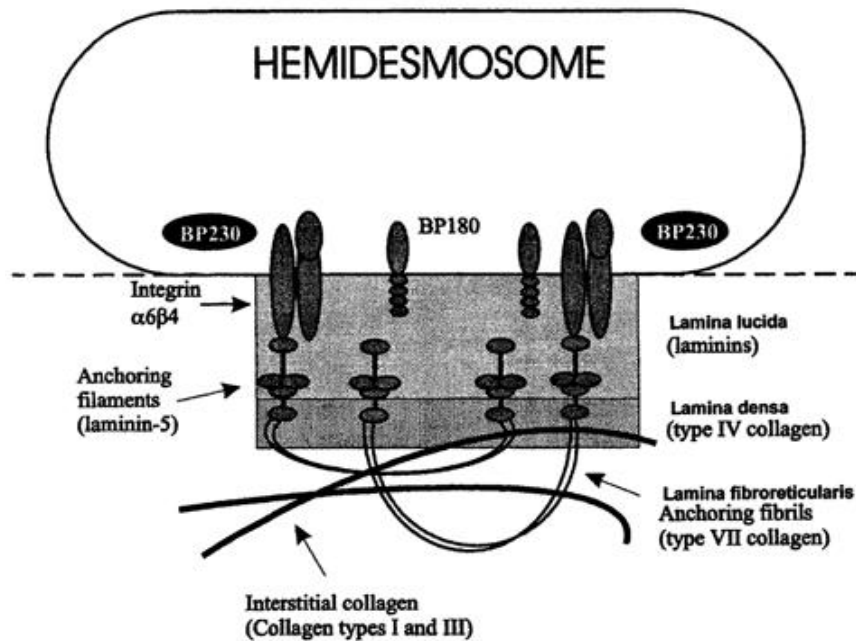
This is the main collagen of the basement membrane. It provides support for epithelial and endothelial cells. Additionally, it functions as a filtration barrier in the kidney. Clinically, mutations in the α -chain can cause Alport syndrome. Type IV collagen contains extensive non-helical portions and does not form fibrils.

Network Collagens: Basal Lamina

Type IV collagen molecules assemble to form network structures. They associate with other molecules and form a sheet on which cells rest, or which surrounds cells.

Filamentous Collagens: e.g. Type VII Collagen

These form the longest triple-helical domains. They are anti-parallel dimers with a small overlap. They are the main constituent of anchoring fibrils. For example, type VII in skin connects the basal lamina to the stroma.



Collagen: Multiple Types Occur in Each Organ, e.g. Skin

The below table shown the location in skin of several types of collagen.

Types of Collagen	Location in skin
Type I	Reticular dermis
Type III	Papillary
Type IV, VII and XVII	Basement membrane
Type VIII	Endothelial cells

Connective Tissue Biology 2: Collagen Diseases

Introduction

Collagen diseases can be due to mutations in collagen genes, abnormalities of post-translational modifications (due to a lack or change of enzymes required for the processing), too much degradation of collagen (caused by ageing or diseases), too much production of collagen (deposition), and nutrient related (leading to a lack of cofactors required for collagen synthesis).

Considerations to take into account include asking which type of collagen is affected; there are 26 types of collagen, the vast majority of which are type I. Therefore, most but not all diseases are due to an alteration in type I collagens. The type of collagen affected dictates which tissue and function is most affected.

If it is a mutation, then which collagen chains are affected, and where in the chain is the mutation? It could be at the C- or N- terminal. The location correlates with the severity. Collagen disease can also be a processing defect, depending on which step in the synthetic pathway is defective. It could also be due to a failure in regulation of collagen levels, meaning that there is too much breakdown or synthesis.

Introduction: Diseases of Collagen Genes

Collagen diseases are incredibly rare, but they are very good at illustrating how disease arises due to genetic defects which may apply to more complicated situations. We can then relate defect to clinical signs and symptoms to biochemical/physiological problems.

Osteogenesis Imperfecta (OI): Intro

This is also known as brittle bone (glass) disease. It is a defect in type I collagen and caused by mutations of a collagen gene. This is a group of disorders and there are four main types, the differences between which are related to where the mutation occurs. It is uncommon, having an incidence of 1 in 20,000 and this incidence is not affected by gender or race.

OI: General Characteristics

OI only affects type I collagen, resulting in fragile bones, especially children. This also results in a short stature. Type I collagen is one of the collagens of skin (the dominant one), resulting in thin skin (specifically a thin dermis), which can tear more easily. In the gingiva, collagen turns over much faster than in other places, meaning that in OI, teeth become wobbly and are more likely to fall out.

Tendons are about 80% type I collagen, meaning that they are weak in OI. Additionally, the cornea which has a large collagen component becomes very thin and a blue sclera can be seen beneath. As well as this, the bones in the ear can be affected, resulting in hearing loss.

OI: Biochemical Considerations

OI has an autosomal dominant inheritance and is found on chromosomes 7 and 17. Type I collagen is made up of 2x $\alpha 1(I)$ genes (heterotrimeric) and 1x $\alpha 2(I)$ gene, so the severity of the disease depends on which gene is affected; the gene which gives two strands, or the other which gives only one. This results in either less collagen, or abnormal (but present) collagen.

OI: Mutations

OI has many mutations; in fact there are over 200 different mutations which can result in OI. Frequently, the mutation is a point mutation in which a glycine is substituted for something else. Glycine is every third amino acid and has a small R group (hydrogen) to fit in the centre of the triple helix. Without the glycine, the triple helix becomes very unstable and does not form properly, resulting in weaker collagen.

There are four main types of mutation. They can:

- Alter the amount of type I pro-collagen
- Alter the structure of the triple-helical domain
- Alter residues of COOH propeptide (as the triple helix forms from the C-terminal)
- Alter chain composition of type I procollagen by decreased production of pro- $\alpha 2(I)$.

OI: Excluded (haploinsufficient) Type

In most cases, as long as one allele is unaltered, no disease is present. However, both alleles must be functional for the correct amount of collagen to be present. In haploinsufficient type (type I), no collagen is produced as one allele is not transcribed (this is known as a null allele). The other allele makes collagen as normal. Therefore, half the amount of collagen is produced, though it is all normal collagen, so there is some degree of functionality. This is an insufficient quantity for normal function.

OI: Included Type

In this case, the mutated gene is expressed, meaning that there is a normal amount of collagen, but a lot of it is abnormal. This mutation is most frequently a single base substitution, usually affecting glycine (in 85% of mutations). The collagen produced is unstable.

OI: Factors Determining Severity

To summarise, severity, depends on which gene, the position of the mutation (near COOH terminal means it will be more severe as this is where the triple-helix forms) and which amino acid is substituted for glycine (and how similar or different it is to glycine).

OI: Result of Amino Acid Substitution

This can slow or prevent the triple –helix from forming. Slow formation can give over-hydroxylation. If the substitution is too severe, this can result in intracellular degradation (i.e., if the triple helix does not form.

OI: Inheritance

OI can be inherited as a faulty gene from a parent. The child will have the same type of OI as the parent, but the severity can be different from the parent. Offspring have a 50% chance of inheriting the gene. However, OI can also be due to a spontaneous mutation; 25-35% of patients have no family history of OI.

Epidermolysis Bullosa (EB): Intro

This is a rare heritable disorder which is characterised by severe blistering of the skin. There are three types: simplex; junctional; and dystrophic. These all have the same or similar symptoms, but the cause of each is different.

EB: Types

- Simplex is characterised by blistering in the epidermis, due to defects in keratin filaments.
- Junctional is characterised by blistering in the dermal-epidermal junction due to defects in laminin (predominantly) but also collagen type XVII.
- Dystrophic is characterised by blistering in the dermis due to mutations in the gene encoding collagen type VII.

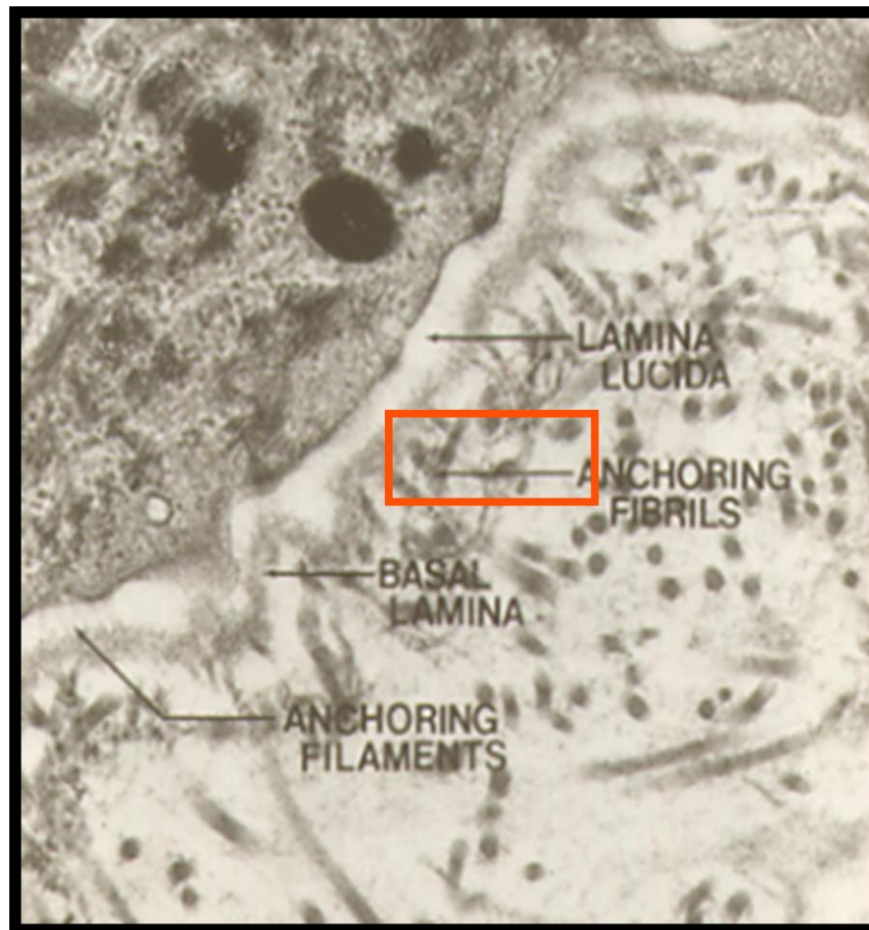
Dystrophic EB (DEB): Types

Dominant dystrophic EB is characterised by blisters on the hands, feet, elbows and knees only. The nails are usually shaped differently and milia (milk spots) may appear on the skin and trunk. DEB may include soft tissue, especially the oesophagus.

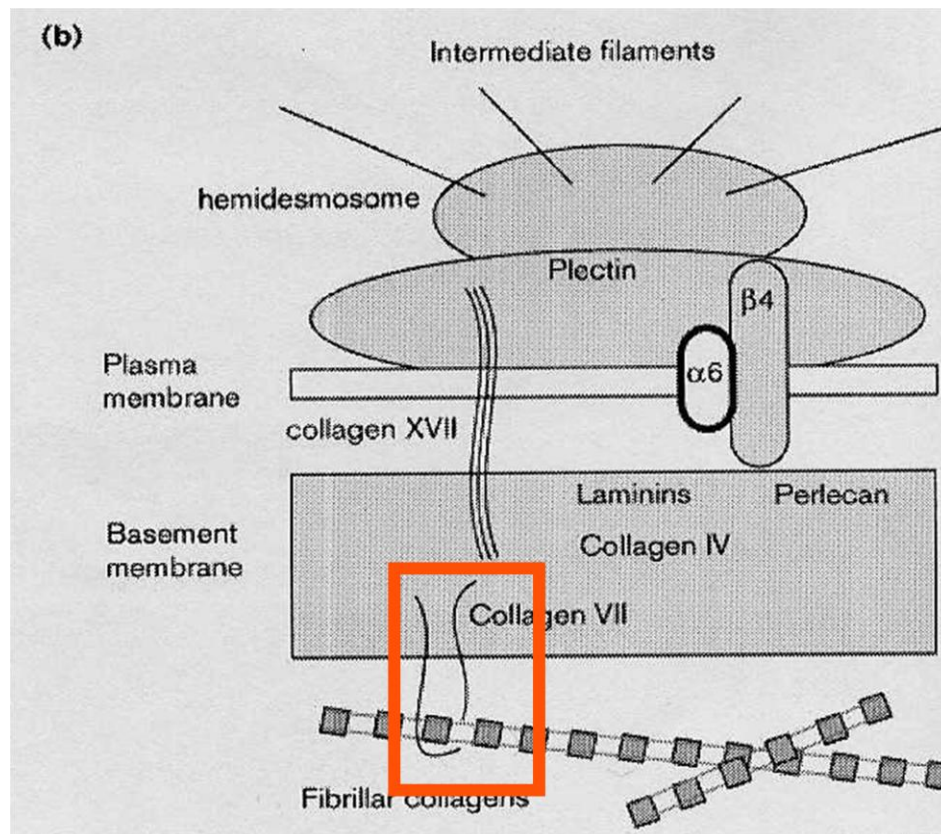
Recessive Dystrophic EB displays with all of the above characteristics as well as eye inflammation, early loss of teeth, blistering inside the mouth and GI tract and fusion of some of the fingers or toes. There is an increased risk of developing skin cancer with recessive DEB.

DEB: Type VII Collagen

DEB can be caused by a mutation in type VII collagen (formed of three identical chains), the anchoring fibril (anchoring the dermis to the epidermis [specifically the basement membrane to the dermis]) which holds the skin together. Mutations can affect any codon; there are over 150 different mutations, which frequently cause premature termination. A “hotspot” for mutations is in exon 73. The location of these anchoring fibrils (type VII) is shown below.



This results in a separation of the two layers, which results in a blister forming. The area boxed in the image below shows where this separation occurs.



EB: Dominant DEB

This is an autosomal dominant condition, though again there are incidences where there is no family history. The mutations cause a decrease in the function of anchoring fibrils, though there are always some present. This causes skin separation at the level of the sub lamina densa of the basement membrane.

EB: Recessive DEB

This is an autosomal recessive inherited condition which can take different forms. It results in the separation of the skin at the level of the sub lamina densa and results in an absence of anchoring fibrils.

Ehlers Danlos Syndrome (EDS): Intro

This is a group of inherited disorders due to defects in collagen synthesis and/or cross-linking. They all result in stretchy skin (hyperextensibility) which bruises and fails to heal, and loose joints (hypermobility), but different types of collagen are affected in all types. A defect in collagen type III can result in arterial problems and colon defects.

Scurvy: Introduction

Scurvy is an acquired disorder, due to a vitamin C deficiency. It prevents hydroxylation of proline to hydroxyproline, giving defective pro- α chains. Due to insufficient hydrogen bonding, a stable triple-helix cannot be formed. Therefore, collagen molecules are degraded inside the cell and consequently pre-existing collagen is not replaced. This leads to fragile tissues, especially those which rely on collagen for strength.

Scurvy: Characteristics

One of the things to be affected is the basement membrane of capillaries, as it turns over relatively quickly. This leads to fragile capillaries which results in subcutaneous and other haemorrhages (blood vessels are easily broken down). Additionally, muscles are weakened due to containing a large amount of connective tissue, and the gingiva of the gums will not be replaced, leading to soft, swollen, bleeding gums and a loosening of the teeth. As well as this, there is very poor wound healing and rupture of scar tissue. After a while osteoporosis will set in too.

Chondrodysplasia: Overview

This is due to abnormalities in collagen types II, IX, X and XI. It affects the endochondral bone formation, resulting in skeletal deformities and dwarfism.

Lathyrism: Overview

This is a diet induced disease which is common in cattle due to the consumption of foods such as seeds of sweet pea which contain β -aminopropionitrile, which chelates copper (binding the ions so that they can no longer function). This is important as copper is a cofactor for lysyl oxidase, which

becomes inhibited. Therefore, collagen cannot be cross-linked, meaning that fibrils are not formed properly.

Osteoarthritis: Overview

This is a loss of articular cartilage and changes in subchondral bone. It affects 40% of people over 65 years old, which is one of the two major risk factors, the other being mechanical load.

Osteoarthritis: Collagen Type IX

Collagen type IX changes with age. There is a reduction in the amount (as cross-linking is affected and degradation is faster than biosynthesis). Additionally, there is a loss of the NC4 domain. This gives alterations to surface properties of collagen fibrils and affects cartilage integrity.

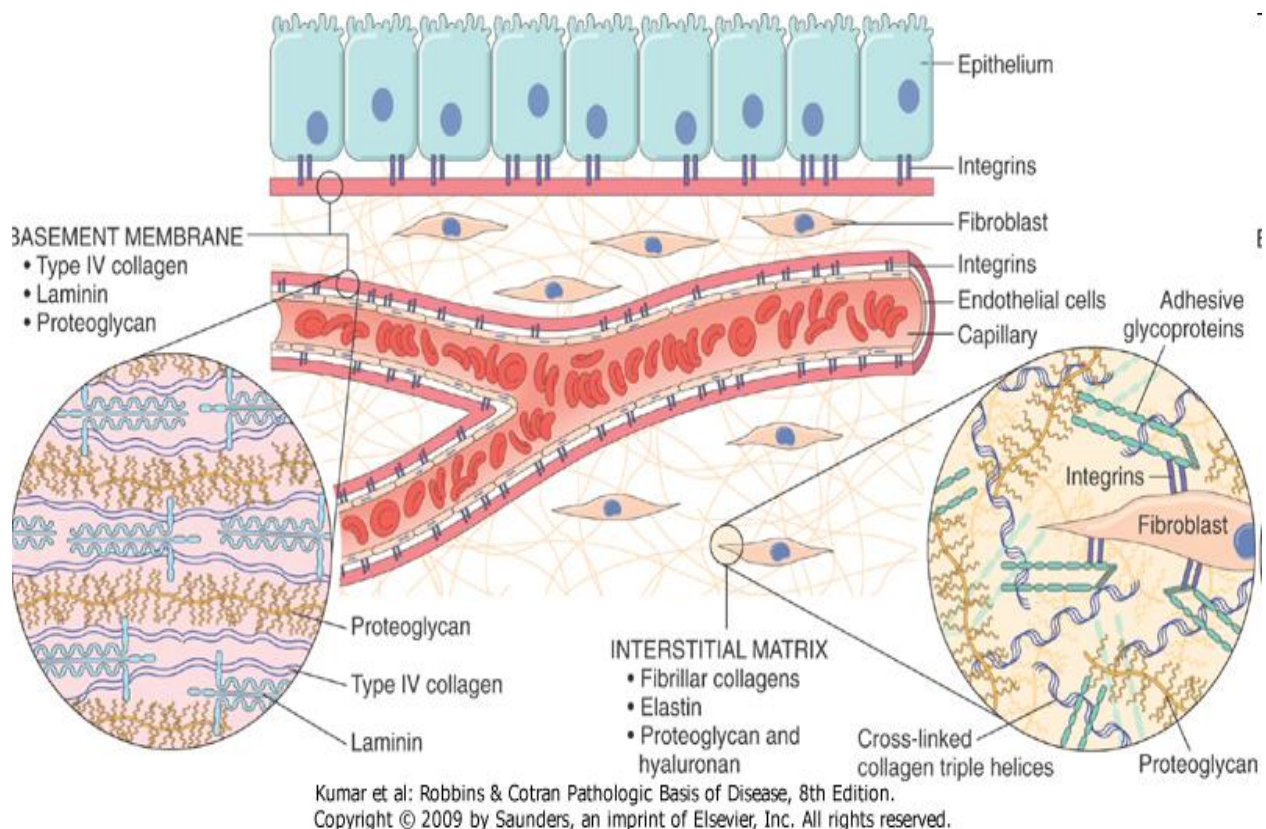
Collagen and Ageing

It is not the purpose of this lecture to look at ageing. One theory of ageing is that the generation of reactive oxygen species causes oxidative damage to tissues. Oxidative changes occur in collagen with age. There is an increase in cross-linking which is accelerated in diabetes and hyperlipidemia. These oxidative changes contribute to the thickening of the basement membrane with age and are implicated in the pathogenesis of diabetes and atherosclerosis. The extracellular matrix of the aorta and major arteries are thicker and more highly cross-linked, decreasing their elasticity and capacity to dilate. This may contribute to an increase of cardiovascular disease with age.

Connective Tissue Biology 3: Non-Collagenous Molecules

Extra Cellular Matrix

One of the outcomes of this lecture is to describe the structure and function of proteoglycans, fibronectin and laminin in the extracellular matrix. The extracellular matrix is shown below.

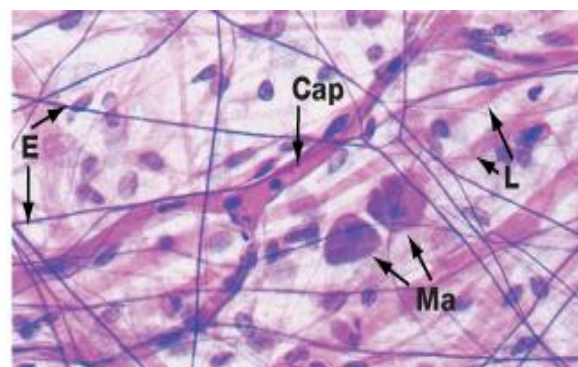


In summary, the extracellular matrix consists of:

- Collagen, which is strong and provides tensile strength whilst resisting stretch.
- Elastin, which provides elasticity (such as that found in skin, the aorta and in ligaments).
- Proteoglycans, which are gel-like and “soak up” force.
- Glycoproteins, which are sticky and adhesive in order to hold fibres and cells together.

Elastic Fibres: Overview

Elastic fibres are formed of elastin and fibrillin. They contribute to the flexibility of the extracellular matrix by stretching without tearing. They allow it to return to its original shape after deformity and are especially important in blood vessels, lungs, ligaments and skin.



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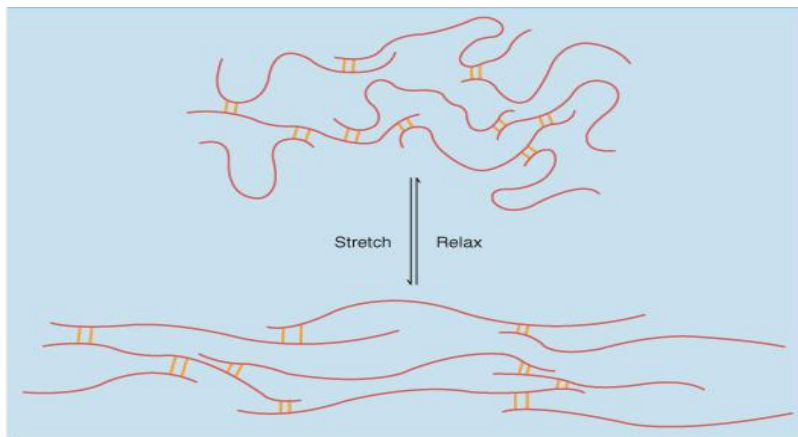
Elastic Fibres: Elastin

Elastin is relatively water insoluble, though is secreted in a soluble form as tropoelastin. It is approximately 750 amino acids long and has no regular secondary structure. Elastin assembles to fibres extracellularly and is held together by cross links. The cross links are mainly via lysine and require lysyl oxidase. These keep the fibres together.

Elastin has an unusual amino acid composition:

- 31% glycine
- 22% alanine
- 14% valine
- 11% proline
- 1% hydroxyproline

Elastin has hydrophobic segments which are the key to its elasticity. It has α -helical segments which are alanine and lysine rich.



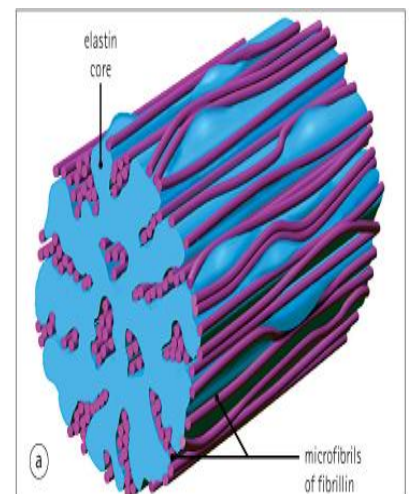
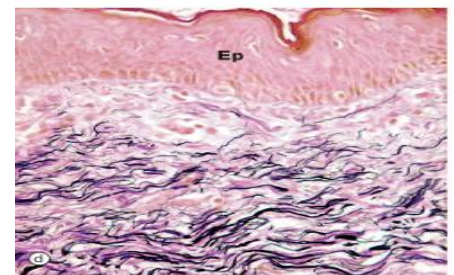
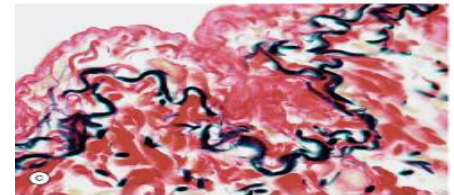
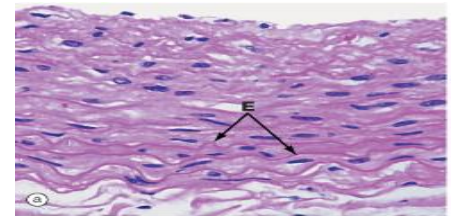
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Elastin Fibres: Fibrillin

Fibrillin is a microfibril with a diameter of 10nm. It is a glycoprotein and surrounds elastin, contributing to stability. It is secreted before elastin and provides a scaffold on which it is deposited (elastin deposits on fibrillin).

Elastin Fibres: Arteries

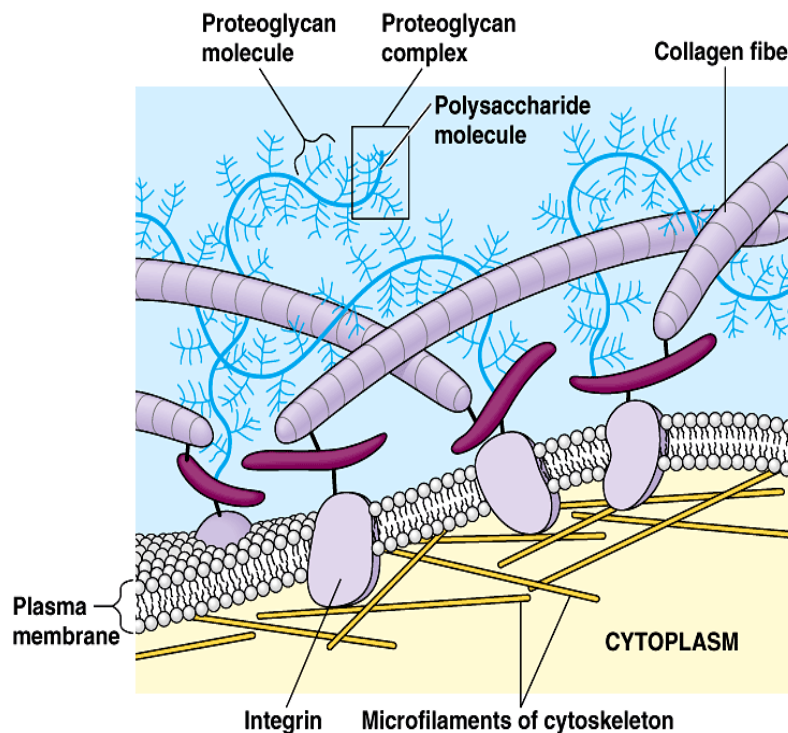
Arteries are made up of an elastin dominant extracellular matrix protein. This makes up 50% of the dry weight of the aorta. Mutations in the elastin gene lead to a deficiency of elastin which results in the narrowing of arteries due to excessive proliferation of smooth muscle cells. Elastin appears to limit smooth muscle cell proliferation.



Elastin Fibres: Marfan Syndrome

Marfan syndrome is a dominant inherited condition due to a defect of fibrillin. Sufferers are unusually tall with long arms and legs, and long, spidery fingers. Their eyes have a displaced lens and the aorta is weak. Additionally, the valves of the heart are “floppy”.

Proteoglycans: Overview



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Proteoglycan is a “ground substance” which makes up a large volume of the matrix.

These molecules are made up of glycosaminoglycans (GAGS) attached to a core protein. They have a rigid, extended, hydrophilic structure and are located in the extracellular matrix and also on the cell surface. Their functions are to form gel in the extracellular matrix which gives matrix packing and resists compressive forces; to lubricate joints; to bind growth factors and extracellular matrix components on the cell surface; and as anticoagulants (heparin).

Proteoglycan: Composition

Proteoglycans are primarily made up of carbohydrate in the form of glycosaminoglycan (95%). These are attached to a core protein, with the exception of hyaluronan (a type of glycosaminoglycan). There are six main classes of proteoglycan:

- Hyaluronan
- Chondroitin sulphate
- Dermatan sulphate
- Keratan sulphate
- Heparan sulphate
- Heparin

Glycosaminoglycan Structure

Glycosaminoglycan is an unbranched polysaccharide composed of a repeating disaccharide. Usually this is an aminosugar (hexosamine) which is attached to uronic acid. Hexosamine is usually sulphated (not hyaluronan) and uronic acid is usually glucuronic or iduronic. Glycosaminoglycan is a highly negatively charged molecule due to its sulphate and carboxyl groups.

Distribution of Main Glycosaminoglycans

Glycosaminoglycan	Sulfation	Protein-linked	Distribution
Hyaluronic acid	no	no	cartilage, synovial fluid, skin, support tissue
Chondroitin sulfate	yes	yes	cartilage, bone, skin, support tissue
Dermatan sulfate	yes	yes	skin, blood vessels, heart
Heparan sulfate	yes	yes	basement membrane, lung arteries
Heparin	yes	yes	lung, liver, skin, mast cell granules
Keratan sulfate	yes	yes	cartilage, cornea, vertebral disk

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Notice that hyaluronan is different in that it is not bound to a core protein and there is no sulphation.

Glycosaminoglycan Properties

Glycosaminoglycan chains cannot fold, leading to extended conformation. Therefore, they occupy large volumes in comparison to their mass. Their negative charge attracts cations, such as Na^+ . They are osmotically active and highly hydrophilic, meaning that they draw water into the matrix and create pressure which resists compressive forces. Additionally, glycosaminoglycan acts as a gel.

Proteoglycan Synthesis

Synthesis of proteoglycan begins with the formation of a core protein. A tetrasaccharide is added which links to serine. Following this, sugars are added one at a time. This requires specific glycosyl transferase enzymes.

Proteoglycan Degradation

This occurs in lysosomes. Lysosomal proteases degrade the protein portion whilst the glycosaminoglycan chains are degraded by the sequential action of different lysosomal acid hydrolases, such as exoglycosidases and sulphatases.

Deficiency of Glycosaminoglycan Degrading Enzymes

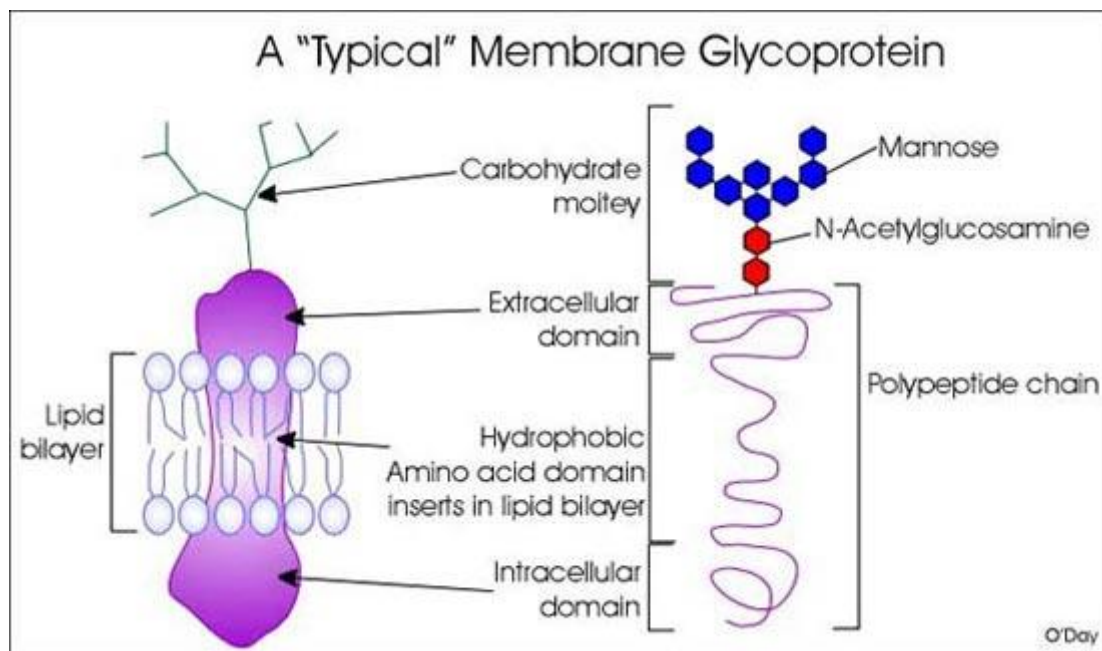
This group of disorders is called mucopolysaccharidoses. It is the loss of a specific enzyme which results in the accumulation of undegraded glycosaminoglycans in lysosomes. This affects all organ systems and is inherited as an autosomal recessive disorder. Common abnormalities arise if

keratin sulphate and dermatan sulphate are affected. These include a short stature, facial coarseness, loss of hearing, joint stiffness and valvular heart disease. If heparin sulphate is affected, neurological and mental abnormalities may present. Collectively, the incidence of mucopolysaccharidoses is 1 per 30,000 births.

Functions of Proteoglycan

Proteoglycan acts as a selective sieve. It is involved in signalling and also in regulation of secreted proteins. It immobilises proteins and sterically blocks their activities, is a reservoir for growth factors and other cytokines, protects from degradation and alters or concentrates protein.

Glycoproteins: Overview



The structure of a glycoprotein is a protein with a carbohydrate attached. Glycoproteins contribute to, but are not limited to, the extracellular matrix. They link different components together and act as a receptor. Additionally, they act as a lubricator and protector. Types common in the extracellular matrix are fibronectin and laminin.

Glycoproteins: General Structure

Glycoproteins are predominantly protein and have short chains of carbohydrate. These are in the form of 12-15 sugar residues, usually D-sugars, but can also be L-fucose, L-arabinose and L-iduronic acid. The percentage of carbohydrate is hugely variable, ranging from 4-60% in some examples. The distribution of CHO chains is variable; they may be evenly spread along the length of the protein, or clustered. Carbohydrate is attached to protein by an N- or O- link.

Glycoproteins in Connective Tissue

Fibronectin is a type of glycoprotein which acts as a “biological glue”. Another type of glycoprotein, laminins, are important in the basement membrane. Other types of glycoprotein are found in small amounts, such as perlecan, tenascin, entactin and thrombospondin.

Fibronectin: Overview

Fibronectin is the most abundant multiadhesive protein. It is found in two forms. The first is soluble in plasma and is involved with blood clotting. The other type is insoluble in the extracellular matrix. Fibronectin is very large with multiple domains. Each is a specific binding site for other proteins.

Fibronectin: Structure

Fibronectin is a polypeptide with functionally distinct domains. These are separated by regions of flexible polypeptide chain. Fibronectin is a dimer (a chemical entity consisting of two structurally similar monomers joined by bonds). The chains may be non-identical. Fibronectin links through S-S bonds at 1 end.

Fibronectin: Map of Binding Regions

Fibronectin has different binding regions for collagen, proteoglycans (heparin), cell surface receptors, fibrin and RGD (integrin binding). This map of binding regions gives the ability to bind to other extracellular matrix components, contributing to the organisation of the matrix whilst giving a stable, interconnected network. Additionally, this map of binding regions attaches cells to the matrix in a “glue” like manner.

Fibronectin: Functions

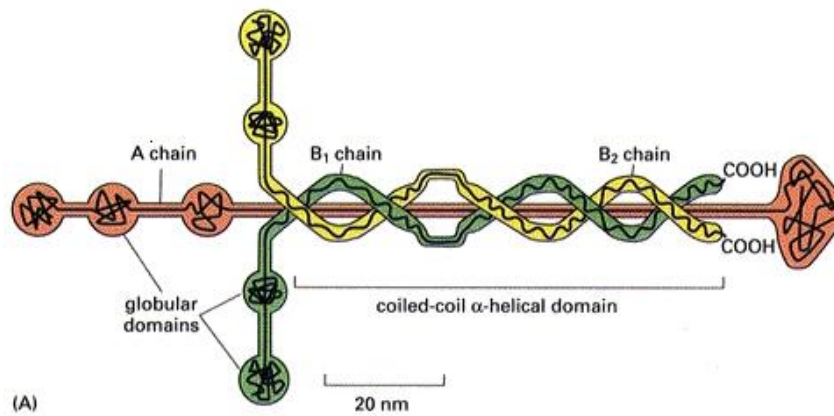
Fibronectin forms a pathway for migrating cells. This is especially important in development and gives the shape of some organs. Additionally, fibronectin functions in cell attraction and migration, as well as wound healing by being incorporated into a clot. This attracts fibroblasts and endothelial cells to the clot and provides the pathway for cell migration.

Laminins: Overview

This is a family of molecules with at least 15 isoforms. Laminins form a cross-shape and influence a cell's migration, growth and differentiation. Additionally, they bind to other laminin and extracellular matrix molecules. They are important in the basement membrane.

Laminin: Structure

Laminin consists of three polypeptide chains, α , β and γ . Each consists of 1500 amino acids. Laminin is cross-linked by disulphide bonds in an asymmetric cross. The long arm of this cross consists of the helical regions of the three chains, whereas the small arms are globular domains interspersed with EGF-like repeats (epidermal growth factor-like repeats).



Laminin: Function

Laminin is a major component of the basement membrane. Its different domains bind to perlecan and nidogen and at least two of them are for cell-binding via integrins (receptors that mediate attachment between a cell and the tissues surrounding it). Some cells proliferate (multiply rapidly) or change shape in response to laminin binding.

Laminin: Basement Membrane

Laminin forms an interconnected network. Its polymerisation initiates formation of the basement membrane, providing it with strength and flexibility. It provides binding sites for cells and other extracellular matrix components, holds the membrane together and links to cells.

Basement Membrane: Overview

The basement membrane provides support for epithelial and endothelial sheets and tubes, and may surround individual muscle, fat and Schwann cells. It regulates access of cells to the interstitial stroma and contributes to the properties of the cells attached to it. It binds to both cells and stroma and stores growth factors and cytokines which are released during degradation.

Basement Membrane: Components

The basement membrane consists of type IV collagen in seven different isoforms. Their structures are homologous but differ in amino acid sequence. The basement membrane also consists of laminin in 15 different isoforms, nidogen (entactin), perlecan, heparin sulphate proteoglycans (which influences the permeability for soluble proteins), and smaller quantities of fifty other proteins.

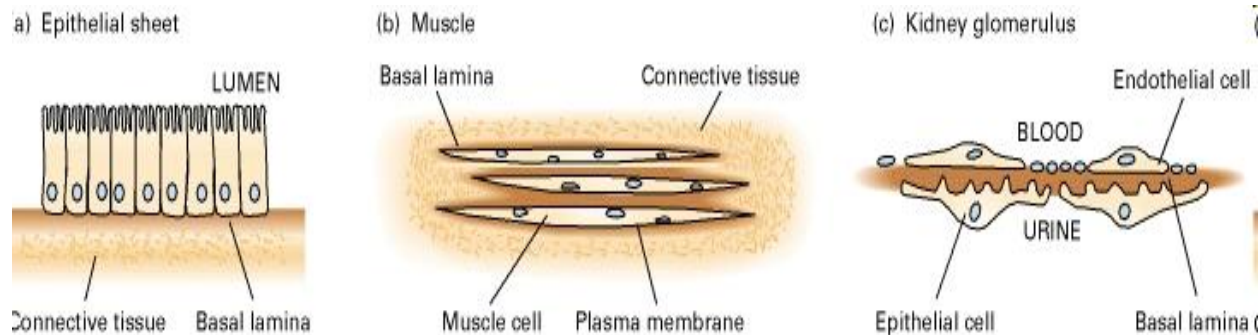
Basement Membrane: Networks

Laminin and type IV collagen each form networks through self-association. The laminin sheet-like structure is initiated by binding to a cell surface receptor. The two networks are linked together via perlecan and entactin (nidogen).

Basement Membrane: Function

The basement membrane allows interactions between the cell and underlying interstitial stroma (the connective, functionally supportive framework of a biological cell, tissue, or organ). It

influences the cell's properties in aspects such as polarity, metabolism, survival, proliferation, differentiation and as a pathway for cell movement. Perlecan absorbs water to withstand compressive force. The basement membrane also prevents interaction of fibroblasts and epithelial cells. Macrophages, lymphocytes and nerve processes can cross it. Examples of basement membranes are shown below.



Basement Membrane: Kidney

Glomerulus acts as a selective filter due to a thicker brim. It prevents the passage of macromolecules from blood to urine. Here, heparan sulphate regulates the passage of charged proteins and retains negatively charged proteins. Most plasma proteins are negative. The basement membrane thickens further in diabetes leading to a leaky glomerular basement membrane and reduced heparin sulphate content. A mutation which can occur in the basement membrane of the kidneys is Alport Syndrome, a hereditary kidney disorder. This is due to a type IV collagen mutation.

Diseases

Corneal clouding may result in macular corneal dystrophy due to the undersulphation of keratan sulphate I proteoglycan. A class of muscular dystrophy associated with a mutation in the alpha-2 chain of laminin-2 prevents normal polymerisation and results in an abnormal basement membrane surrounding the muscle fibre. Another disease is junctional epidermolysis bullosa which is due to a mutation in laminin (see the collagen disease lecture).

Connective Tissue Biology 4: Articular Cartilage Structure, Function and Metabolism

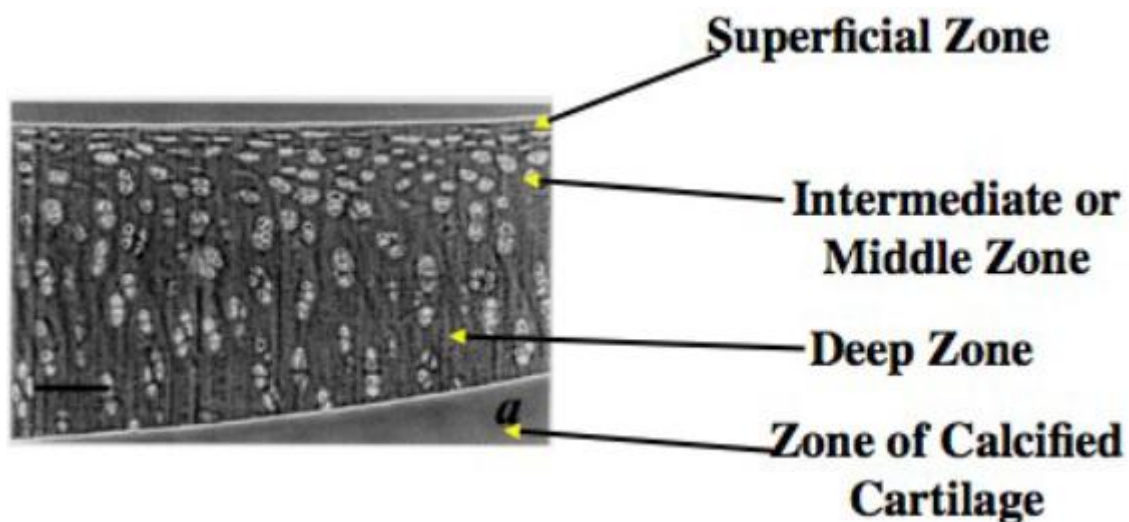
Articular Cartilage Biology

Articular cartilage is a tissue that occurs at the end of bones in all diarthrodial joints. During growth and development it is quite cellular (with many dividing cells) and is relatively unorganized at the ultrastructural level. At skeletal maturity, it becomes a more organized structure where the cells (chondrocytes) are usually found as single cells within lacunae surrounded by an extensive extracellular matrix. Under normal circumstances (i.e. no pathology), these cells do not divide during adult life. These chondrocytes maintain the components of the cartilage extracellular matrix through normal turnover (biosynthesis & degradation) of their component macromolecules.

Adult articular cartilage is Hypocellular, Aneural, Avascular with no Lymphatic system. It is also an interesting tissue/organ in that it is not surrounded by a Basement Membrane.

Morphology

Cells in healthy articular cartilage are organized into four zones: the superficial zone, a single layer of flattened cells that are aligned parallel to the surface; randomly organized intermediate or mid-zone cells; deep-zone cells aligned in columns of lacunae; and a zone of calcified cartilage. The alignment of these cells within the matrix is maintained by the collagen fibre organization termed Benninghoff Arcades



Cartilage composition

The Major Components of Cartilage are Collagens, Proteoglycans and Water. There are also lesser amounts of matrix glycoproteins and plasma proteins.

WET TISSUE	DRY TISSUE
Water - 70%	Collagens - 75%
Collagens - 20%	Proteoglycans - 22%
Proteoglycans - 7%	Other Proteins - 3%
Cells - 2%	
Other Proteins - 1%	

Cartilage Collagens

- Type II (94%) - Major fibrillar collagen. Gives tensile strength.
- Type VI (1%) - In the pericellular (cell associated) matrix - in lacunae
- Type IX (2%) - Fibril-associated collagen (on the outside) resists shear.
- Type XI (3%) - Involved in fibril nucleation (at centre of Type II fibrils).

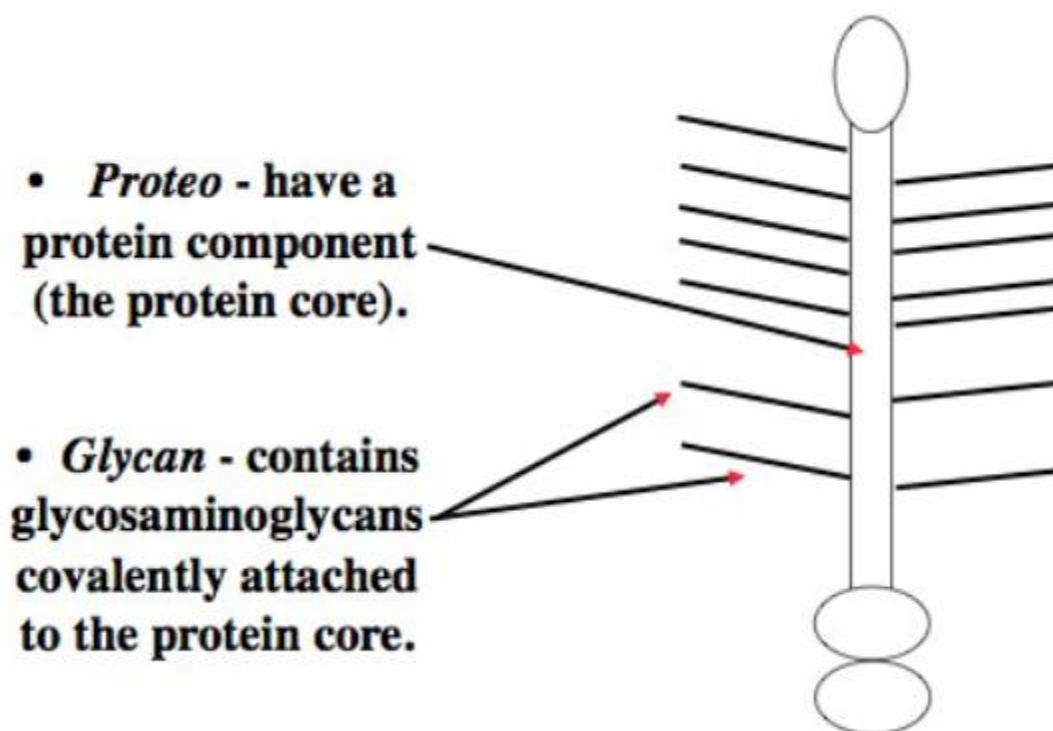
Collagen structure and function

Fibrillar collagens (type II & XI) form an organized heterotypic collagen fibre meshwork that gives the tissue its tensile properties. Type XI is involved in fibril nucleation and is located within the "heterotypic collagen fibrils" in cartilage. The fibril-associated collagen (Type IX) adds to this tensile strength by possibly cross linking the fibrils or acting to resist shear between fibrils (like 'barbs' on barbed wire). Type VI is a globular collagen (it does not have not much triple helix and therefore does not form fibrils). It forms a loose fibrillar meshwork in lacunae around the cells (chondrocytes) helping in their protection against mechanical forces.

Cartilage Proteoglycans

There are two types of cartilage proteoglycan: large aggregating proteoglycans (such as aggrecan); and small proteoglycans (such as decorin, biglycan and fibromodulin).

Proteoglycan Structure

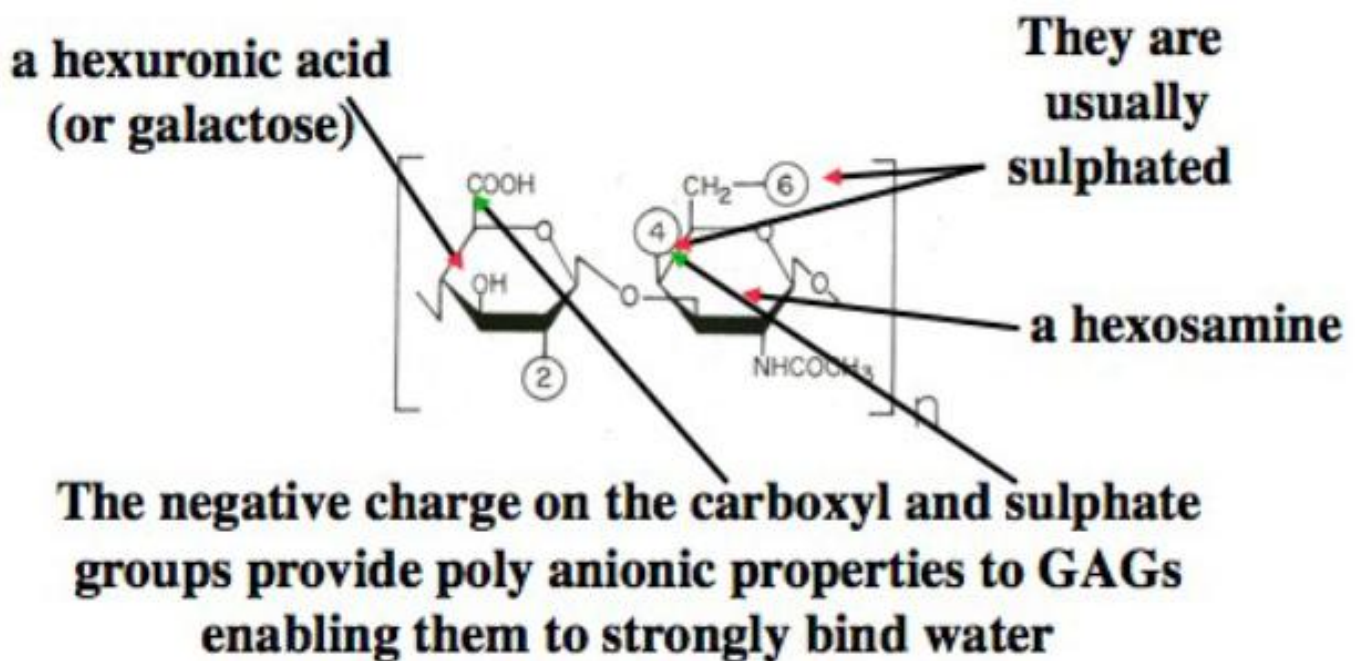


Proteoglycan core proteins are complex proteins with multi-functional domains. These domains are involved in specific protein - protein, protein - carbohydrate (e.g. GAG) and protein - cell interactions that help to hold together the tissue, or its components.

Glycosaminoglycan (GAG) chains are covalently bound to the protein core polypeptide through the hydroxyl groups of Serine or Threonine or the amine group of Asparagine amino acid residues.

Glycosaminoglycan structure

These are long polysaccharides composed of repeating disaccharide units. The disaccharide units always contain a hexosamine (usually N-acetylated) and a hexuronic acid (contains a -COOH at C-6 of the sugar) or galactose. These disaccharides are usually sulphated in at least one of the sugars. The presence of these sulphate groups and the carboxylate groups give these molecules a very high negative charge (they are long chain polyanions). The polyanionic glycosaminoglycans have a high affinity for water.



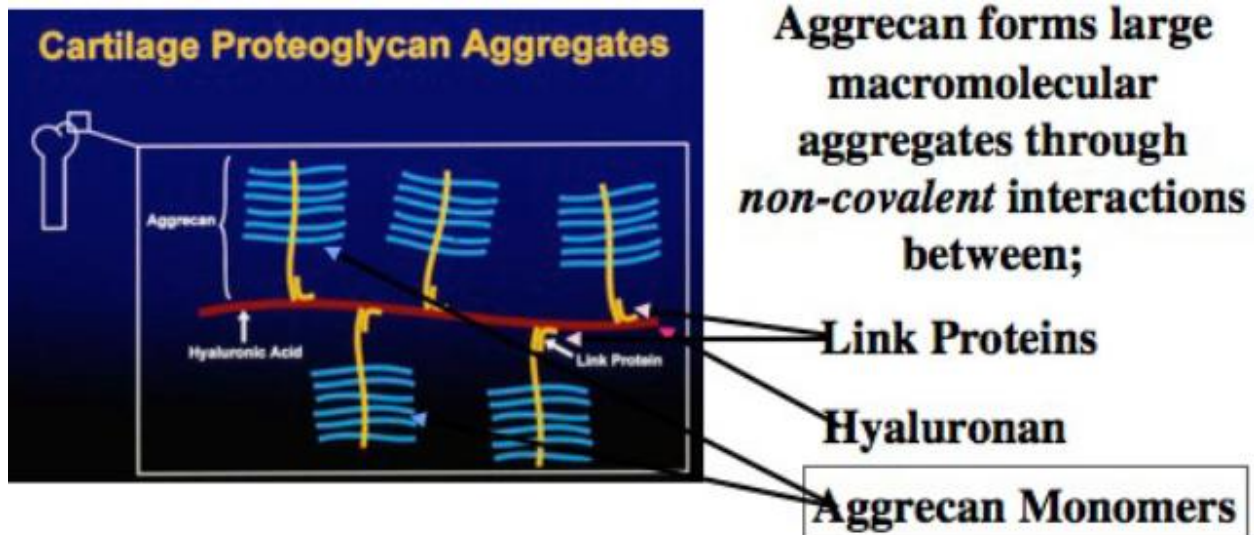
Aggrecan

Aggrecan (monomer) is the major proteoglycan present in cartilage. It has a core protein of 50,000 dalton with several distinct structural domains. At the N-terminal it has globular domains, one of which (the hyaluronan binding domain) specifically binds non-covalently to hyaluronan (HA - also called hyaluronic acid which is a long linear glycosaminoglycan). In addition, there is a long extended protein domain called the glycosaminoglycan attachment domain where two specific types of glycosaminoglycan are attached - chondroitin sulphate (around 100 chains) and keratan sulphate (around 50 chains).

The molecular mass of each aggrecan monomer is very high; approximately 2.5 million dalton. The covalent attachment of large numbers of these polyanionic glycosaminoglycans on to the aggrecan protein core gives the aggrecan monomers a very high affinity for water.

Proteoglycan Aggregate Formation

The aggrecan monomers (30 - 50 of them) form large macromolecular aggregates by aggregating with a single hyaluronic acid (HA) glycosaminoglycan chain through their globular hyaluronic acid binding domain at the N-terminus of each aggrecan monomer (See Slide 21 & 22). This non-covalent association is stabilized by interaction with a small glycoprotein called link protein that also non-covalently associates with both the aggrecan monomer and the hyaluronic acid. These non-covalent interactions between the three molecules (aggrecan monomers, HA and link protein) are very strong and extremely stable.



Proteoglycan Aggregate Assembly

Each of the components of the aggregates are synthesized separately and assembled in the cartilage extracellular matrix. The resultant macromolecular aggregates (over 100 million dalton) become entrapped within the collagenous fibre meshwork. Their strong water binding properties create high osmotic pressures that cause the aggregates to expand in order to maximally repel the polyanionic glycosaminoglycans. This expansion is resisted by the collagen fibre meshwork. The proteoglycans essentially become immobilized (they form a gel) within the tissue.

Small Interstitial Proteoglycans

These are small molecular weight proteoglycans compared to aggrecan (70-120,000 dalton compared to 2.5 million dalton for an aggrecan monomer). They are commonly found in most connective tissue extracellular matrices. There are three species found in articular cartilage:

- Decorin - contains one chondroitin sulphate (CS) or dermatan sulphate (DS) glycosaminoglycan chain covalently attached to a core protein of ~40,000 dalton
- Biglycan - contains two CS or DS chains covalently bound to a ~40,000 dalton core protein. It is usually found in the pericellular matrix of connective tissues (the lacunae of cartilage).
- Fibromodulin - contains 3 - 5 keratan sulfate (KS) chains covalently bound to a ~50,000 dalton core protein. There are also tyrosine (Y) sulphate residues at the N-terminal of the molecule.

Function of small proteoglycans

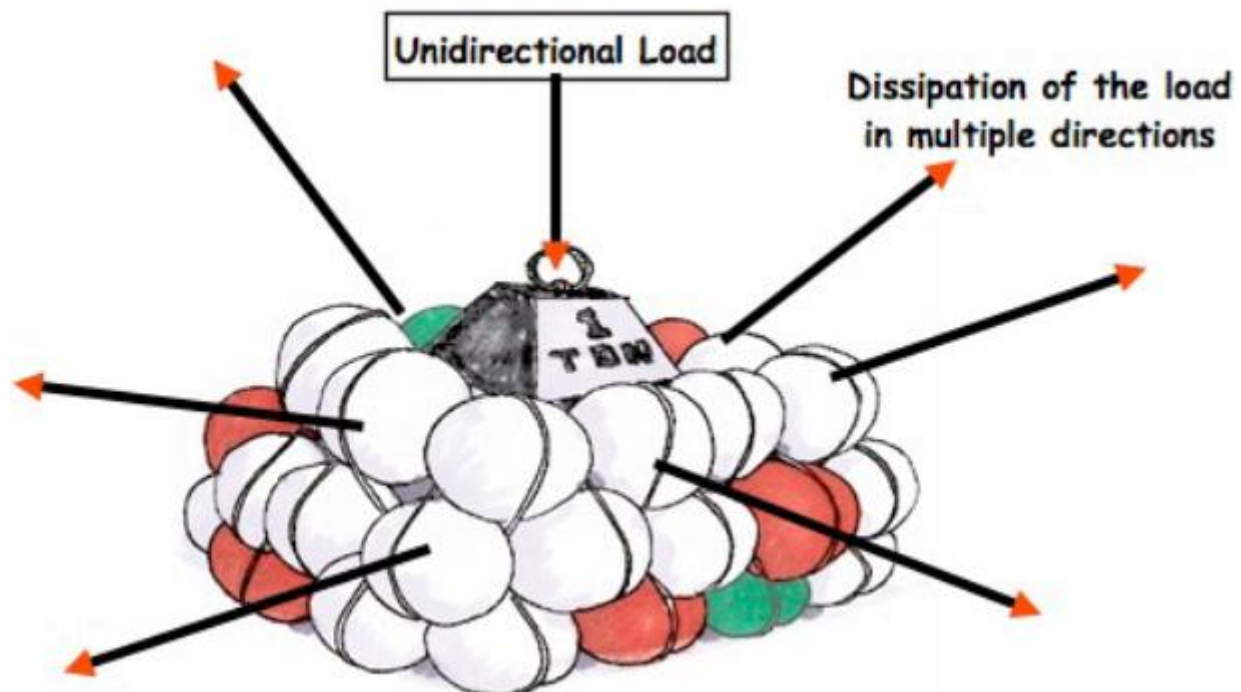
Both decorin and fibromodulin regulate collagen fibrillogenesis. They do this by binding to specific regions of the collagen fibrils as they form larger fibres and thus control the size and resultant organization of the collagen fibre meshwork within a given tissue. Decorin can also bind growth factors such as TGF- β at specific sites on its core protein thereby sequestering (storing) them within the extracellular matrix of cartilage. Biglycan is always found closely associated with and around cells (for example, in the lacunae of cartilage). Biglycan also binds to a variety of growth factors and facilitates their presentation to cells at their specific cell surface receptors.

Other Proteins

- Matrix glycoproteins - these form secondary meshwork e.g. matrilins
- Plasma proteins - plasma filtrate

Cartilage Proteoglycan Aggregate Function

The function of large aggregating proteoglycans in cartilage is to bind water strongly (through its hydrophilic glycosaminoglycans) and thereby resist its flow from the tissue when compressive loads are administered to the cartilage surface during joint articulation. They do this by resisting the flow of water from the tissue when it is compressed. The cartilage surface becomes deformed in loading and water is slowly forced out of the tissue. Because water is incompressible its movement helps dissipate a unidirectional load into all directions throughout the tissue. Once the load is removed the water rapidly goes back into the tissue and rehydrates the hydrophilic proteoglycans.



Synovial Joint Lubrication

The biphasic nature of cartilage with its fluid phase interacting with a solid phase of collagens and proteoglycans provides the potential for surface lubrication through the flow of interstitial fluid from the cartilage at the point of contact at the leading and trailing edges of the point of loading during articulation. In the past, much of the lubricating properties of synovial joints has been attributed to the presence of hyaluronan (hyaluronic acid) in the synovial fluid. However, past and recent research has now identified Lubricin (alternatively known as Superficial Zone Protein/Proteoglycan – SZP) as the major macromolecule providing lubrication and other important properties to the surface of articular cartilage.

Lubricin is synthesized by the surface zone chondrocytes. It has many functional domains; the main domain providing lubricating properties is the mucin-like domain that contains many negatively charged O-linked oligosaccharide structures. These mucin-like oligosaccharides prevent cell attachment to the surface of articular cartilage; i.e. their presence provides chondroprotection/cytoprotection at the cartilage surface as well as lubrication properties. Joint inflammation causes a decrease in lubricin biosynthesis and a loss of lubricating properties at the joint surfaces.

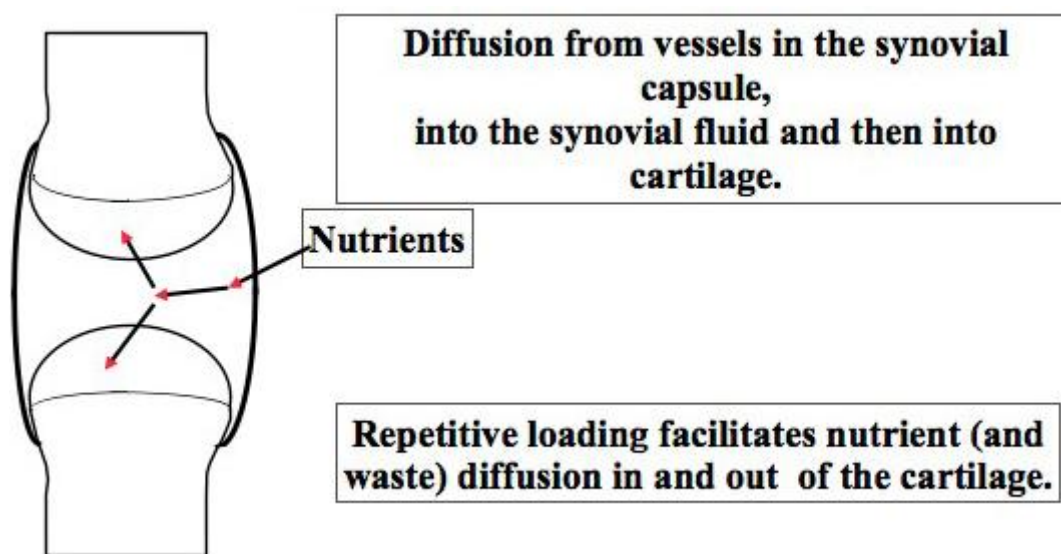
Lecture Objectives and Review Questions

1. Know the basic biology and morphology of articular adult cartilage
 - What are the basic biological characteristics of articular cartilage?
 - What are the morphological features of the cellular and matrix organisation of adult articular cartilage?
2. Know the major components of articular cartilage
 - What are the collagen types found in healthy adult articular cartilage?
 - What are the basic types of proteoglycans found in articular cartilage?
 - What are the general characteristics of glycosaminoglycans?
 - What are the components of proteoglycan aggregates in cartilage?
3. Know the functions of collagen(s) and proteoglycans in cartilage
 - What is the primary function of type II collagen (the major fibrillar collagen) present in articular cartilage?
 - What is the primary function of the large proteoglycan aggregates in articular cartilage?
 - What is the function of the small proteoglycans in articular cartilage?
 - How so the composite functions of the articular cartilage collagens, proteoglycans and water collectively provide its unique physiological functions?
4.
 - a. What is the major macromolecule responsible for synovial joint lubrication?
 - b. What are the biochemical components that provide the lubrication properties and cytoprotection?

Connective Tissue Biology 5: Articular Cartilage Metabolism in Degenerative Joint Diseases

Articular Cartilage in Growth, Development & Ageing

During growth and development, articular cartilage is much more cellular than adult cartilage (in relation to the extracellular matrix) and also the four zones of cartilage cell morphology seen in adult cartilage is not apparent. Upon reaching adulthood, the physes of the growth plates close as indicated by the formation of a 'tidemark' in the zone of calcified cartilage. This closure restricts the access of the articular cartilage chondrocytes to nutrients from the subchondral blood supply. Thus, cartilage nutrition (and normal tissue homeostasis) relies on diffusion of nutrients from the blood vessels in the synovial capsule into the synovial fluid and then into the cartilage. Movement of tissue water during reversible compressive movement across the articular surfaces facilitates the passage of nutrients (and waste products) in and out of the cartilage.



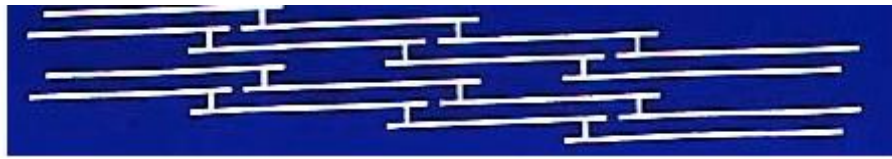
In healthy adult cartilage, the chondrocytes do not generally undergo cell division. During ageing the chondrocytes are responsible for maintaining tissue homeostasis (an equal balance between synthesis on new, and degradation of 'old', cell and extracellular matrix molecules required to keep the cartilage functioning normally).

Biochemical Changes in Articular Cartilage with Ageing

Cell metabolism slows down and cell number decreases; collagens and other matrix proteins (such as matrilins and cartilage matrix protein) become cross-linked (see below); type IX collagen synthesis and deposition is significantly reduced; Aggrecan changes in its biochemical composition (there is an increase in the relative proportion of keratan sulphate to chondroitin sulphate glycosaminoglycans (GAGs; the CS GAGs become shorter and the ratio of 6-sulphated to 4-sulphated CS on cartilage aggrecan increases); and there is an increase in the accumulation of degradation products (catabolites) of the cartilage matrix macromolecules.

The cross-linking occurs between modified hydroxylysine residues in the collagen molecules and through the enzyme activity of transglutaminases that form covalent bonds between lysine and glutamine residues on specific adjacent matrix proteins. This increase in crosslinking of matrix

molecules makes the cartilage more brittle (with ageing) and therefore more subject to damage by mechanical forces.



Cartilage turnover

Once skeletal maturity is reached, the metabolism of the chondrocyte drops dramatically. The main function of the chondrocyte in healthy adult cartilage is to maintain the constituents of the cartilage extracellular matrix so that it can continue to perform its physiological function of resisting compression under load during joint articulation. A balance between turnover (synthesis versus degradation) of the cartilage matrix macromolecules is maintained. Compared to other molecules (such as liver or serum proteins) the turnover of the cartilage collagens are very slow (taking years) and the proteoglycans, several months in healthy tissue. The catabolism of cartilage extracellular matrix macromolecules (collagens and proteoglycans) is performed by matrix proteinases that specifically degrade specific collagens, proteoglycans and matrix proteins.

Matrix Proteinases

Matrix proteases are proteolytic enzymes that are capable of degrading extracellular matrix molecules at a physiological pH. Common members of this group are the Matrix Metalloproteinases (MMPs) and also, the only recently discovered "aggrecanases". There are over 20 members of the MMP gene family and they are responsible for degradation of matrix macromolecules (e.g. collagens, elastin, fibronectin etc,) in many connective tissues. The "aggrecanases" belong to a recently discovered gene family (over 10 members) denoted ADAMTS which is an acronym standing for A Disintegrin and Metalloproteinase with Thrombospondin motifs. There are two "aggrecanases" denoted ADAMTS-4 & ADAMTS-5. The aggrecanases degrade aggrecan at specific sites within aggrecan core protein.

Both the MMPs and ADAMTS families are "Metalloproteinases" because they require Zinc and/or calcium ions for their activities. The zinc binding to these proteins is through a specific amino acid sequence motif of ..HExxHxH.. where the Histidine (H) residues specifically chelate the zinc cation and thus facilitate proteolytic cleavage of specific peptide bonds within matrix macromolecules.

Matrix Proteinases in Cartilage Metabolism

Matrix proteinases are responsible for the degradation of cartilage matrix molecules in normal turnover and they are also responsible for the increased catabolism of cartilage matrix molecules in degenerative joint diseases (e.g. arthritis). The two major classes of matrix proteinases are involved in cartilage metabolism; they are; the Matrixmetalloproteinases [MMPs] and the "aggrecanases" [ADAMTS-4 & ADAMTS-5(11)]. The MMPs are primarily involved in catabolism and slow turnover of the cartilage collagens and other matrix proteins (but not aggrecan). The "aggrecanases" are specifically involved in aggrecan catabolism (degradation) in cartilage.

The Aggrecanases

The aggrecanases are two of the ADAMTS gene family members that are expressed in cartilage (and other body tissues) and have been denoted ADAMTS-4 and ADAMTS-5 (or more simply aggrecanase-1 and aggrecanase-2). Unlike MMPs (see below) they are secreted as active enzymes and proteolytically degrade cartilage aggrecan at specific sites within its core protein (i.e. they are called "aggrecanases" because they very specifically cleave aggrecan at a site, within its interglobular domain, between a Glutamic Acid residue (E) and an Alanine residue (A)). Proteolytic cleavage at this site (and others within the aggrecan core protein) causes the release of the glycosaminoglycan-binding domains of aggrecan from the cartilage (the parts of aggrecan that bind and immobilise water within the cartilage extracellular matrix). Loss of these glycosaminoglycan-binding domains reduces the ability of cartilage to bind water and thereby compromises its function of resisting mechanical loading in joint articulation.

Matrix Metalloproteinases (MMPs) in Articular Cartilage

- (i) The Collagenases (MMP-1 & MMP-13): They have specificity for the fibrillar collagens (II, IX & XI) where they cleave the molecules in their triple helix domains. This cleavage disrupts the helix and denatures the molecule.
- (ii) The Gelatinases (MMP-2 & MMP-9): These have specificity for the denatured fibrillar collagens (created after MMP-1 or MMP-13 proteolytic digestion of the fibrils) and also the non-fibrillar collagen (type VI).
- (iii) The Stromelysins (e.g, MMP-3 & -10): These have specificity for degrading the cartilage matrix proteins (not aggrecan) and they can also "activate" the other matrix metalloproteinases (see below).

The Properties of Matrix Metalloproteinases:

- (i) They are secreted and stored in the extracellular matrix as zymogens (inactive enzymes);
- (ii) They require Zinc and/or calcium for their activity (thus the term *metalloproteinase*);
- (iii) They are activated by the removal of a pro-peptide domain from the N-terminal of the zymogen, Removal of this peptide causes conformational changes in the enzyme that brings the catalytic site and the Zinc binding domain into the right position to cause cleavage of specific peptide bonds.

Arthritis

External factors cause an alteration in the balance between the synthesis and breakdown of the cartilage matrix macromolecules which lead to the onset of degenerative joint diseases (such as arthritis). These can occur from autoimmune disorders that have their "cause(s)" in non-articular tissues (e.g. rheumatoid arthritis) or result from disruptions to joint mechanics caused by several factors such as genetic predispositions (e.g. congenital hip dysplasia), trauma (e.g. anterior cruciate ligament rupture in the knee joint) and other factors (e.g. obesity).

In **Rheumatoid Arthritis (RA)**, the presence of immune complexes in the synovial joint causes an influx of inflammatory cells into the synovial joint space. These cells release inflammatory cytokines [e.g. interleukin-1 (IL-1), tumour necrosis factor-alpha (TNF- α)] which cause synovial proliferation and induce the synthesis, secretion and activation of matrix proteases (proteolytic enzymes) that degrade the cartilage matrix molecules.

The onset of **osteoarthritis (OA)** is usually related to an alteration in the mechanical stresses applied to the articular cartilage that often occurs as a result of injury or trauma to the joint. (e.g. anterior cruciate ligament rupture in rugby & football players or skiers). After the acute injury, its progression is usually non-inflammatory in its early stages. The altered mechanical stresses to the cartilage cause changes in the cartilage metabolism. There are attempts to repair and remodel the cartilage so as to alter its biochemical composition (e.g. collagen to proteoglycan content) to compensate for the changes in load distribution. This attempted remodeling further compromises the ability of the cartilage to resist mechanical loads. In addition, the altered mechanical stresses induce the chondrocytes to make cytokines (interleukin-1) that, in turn, cause the synthesis, secretion and activation of matrix proteases that degrade the cartilage matrix molecules. This leads to loss of function and destruction of the articular cartilage eventually leading to the need for joint replacement surgery.

Cartilage Destruction in Arthritis

Several biochemical changes in the composition and metabolism of the cartilage occur before any overt signs of cartilage degradation are apparent. They are;

- (i) *Cartilage exposure to increased cytokine levels:* This occurs from either inflammatory cell infiltration (e.g. macrophages and neutrophils) into the synovial joint (i.e. in RA) or their autocrine synthesis by the cartilage chondrocytes due to changed mechanical stresses on the articular cartilage joint surfaces (i.e. in OA). This exposure to cytokines (e.g. interleukin-1 or tumor necrosis factor- α) causes changes in the metabolism of the chondrocytes such that there is increased turnover (synthesis & degradation) of the cartilage matrix molecules i.e. synthesis and activation of matrix proteases. Thus, although RA and OA have very different etiologies (causes) they have some common mechanisms and pathways which eventually leads to tissue destruction - see below).
- (ii) *The chondrocyte's attempt to repair and/or remodel the cartilage:* In RA, the cytokines (IL-1 & TNF- α) released from the inflammatory cells infiltrating the synovial joint causing the degradation of cartilage aggrecan and collagens by matrix proteases ("aggrecanases" and MMPs, respectively). In OA, there are increases in mechanical stresses to the articular cartilage. This stimulates increased cytokine synthesis by the chondrocytes and results in minor degradation of matrix molecules including some of the collagen fibrils which causes a slight swelling of the cartilage (i.e. there is an increased water content). The increased loss of matrix proteins in this attempted remodelling is initially compensated by an increase in their turnover (i.e. both their biosynthesis and degradation increases but they have shorter half-lives). However,

increased exposure to cytokines (e.g. IL-1 & TNF- α) eventually causes an inhibition of cartilage aggrecan biosynthesis and an increase in its catabolism by "aggrecanases".

- (iii) *Proteoglycan loss from the superficial (surface) zones of the cartilage:* This is seen histologically as a loss of cationic dye staining (e.g. Toluidine blue or Alcian blue) - **see Slides 21 & 26** . This loss of proteoglycan (i.e. caused by aggrecanase removal of the glycosaminoglycan binding domains of aggrecan - thus loss of histological staining) compromises the ability of the cartilage to bind and immobilise water within the tissue and thereby resist compressive loads. This makes the surface of the cartilage susceptible to mechanical disruption and the occurrence of cartilage surface fibrillation.
- (iv) Further activation of matrix proteases: Exposure to cytokines (e.g, IL-1 & TNF- α) also causes the activation and increased synthesis of MMPs (collagenases, gelatinases & stromelysins) by the chondrocytes. This occurs after there has been significant loss of aggrecan from the cartilage. This increase in MMP activity causes increased degradation of the collagen fibre meshwork which severely and eventually irreversibly destroys the collagenous architecture and compromises the tensile properties of the cartilage (Catabolism of aggrecan by aggrecanases has already reduced the cartilage's ability to bind water and resist compressive loads). Collectively, this results in a wearing away of the cartilage (from both enzymatic and mechanical destruction) to eventually expose the underlying bone. This loss of cartilage leads to joint space narrowing which is apparent on X-ray analysis. Ultimately, the RA or OA patient requires total joint replacement surgery.
- (v) Chondrocyte "cluster" formation (Slides 26 & 27): In the later stages in the development of arthritis there are also cellular changes in the chondrocyte phenotype and morphology (i.e. there is chondrocyte dedifferentiation). Here the cells start to divide in their lacunae and become hypertrophic (i.e. form "cell clusters"). These chondrocytes attempt to repair the tissue damage but this inevitably fails.

Treatments for Arthritis

- (i) Steroid treatment is used to reduce the infiltration of inflammatory cells into the synovial joint in RA.
- (ii) Non-Steroidal Anti-Inflammatory Drugs (NSAID's) are used to reduce inflammatory mediators (e.g. prostaglandins) that cause the synthesis of inflammatory cytokines and cause joint pain in RA and late-stage OA.
- (iii) Anti-cytokine antibodies (e.g. anti TNF- α antibodies) that are used to neutralize their local joint effects in the treatment of RA;
- (iv) Surgical options - synovectomy for RA patients; osteotomy to change mechanical distribution to joints in early OA; total joint replacement for end stage OA & RA patients; joint reconstruction for ACL tears.

- (v) Dietary intake of n-3 (omega-3) fatty acids (i.e. those present in Cod Liver Oil) that reduce the synthesis and expression of aggrecanases and enzymes (cyclooxygenases and lipoxygenases) that produce inflammatory mediators (prostaglandins, leukotrienes and cytokines).
- (vi) Development of drugs that can specifically target and inhibit the synthesis and/or activation of matrix proteases (e.g. the aggrecanases in early stages of arthritis, and/or the collagenases in later stages of the disease) or drugs that inhibit the signalling mechanisms of the inflammatory cytokines that cause upregulation of factors inducing cartilage destruction in arthritis.
- (vii) The use of stem cells (chondrogenic progenitor cells) or autologous chondrocytes to repopulate and repair cartilage defects that lead to degenerative joint disorders.

Learning Objectives and Review Questions

1. Know the morphological and biochemical changes that occur with ageing.

- (a) What are the cell and tissue morphology changes with age?
- (b) What structural changes occur to the collagens and matrix proteins in articular cartilage ageing?
- (c) What biochemical changes occur to cartilage aggrecan in ageing?

2. Know the basic classes and functions of matrix proteases in cartilage metabolism.

- (a) What are the general classes of matrix metalloproteinases (MMPs)?
- (b) What are the general properties of MMPs?
- (c) What class of matrix proteases do the two Aggrecanases belong to?
- (d) What are the general properties of the Aggrecanases?
- (e) What is the Interglobular Domain (IGD) cleavage site of Aggrecanase cleavage of aggrecan?

3. Know that the main events that lead to cartilage destruction in arthritis

- (a) What are the early events that occur in the pathogenesis of arthritis?
- (b) What are the matrix changes that lead to early fibrillation of cartilage in arthritis?
- (c) What are the changes in metabolism of cartilage that lead to joint space narrowing in the later stages of arthritis?

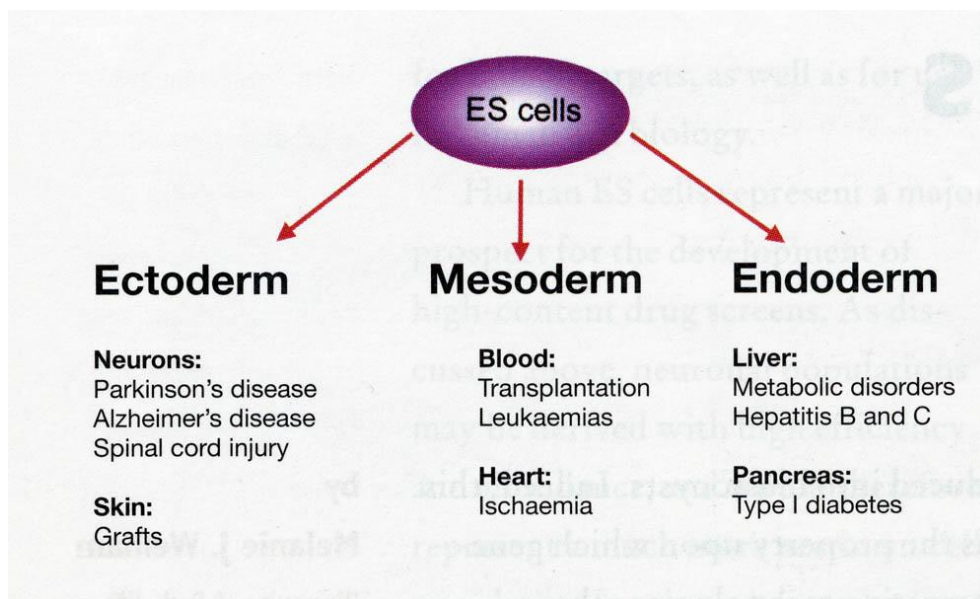
Connective Tissue Biology 6: Tissue Engineering & Regeneration

Tissue engineering and tissue regeneration is involved in a wide range of clinical applications, such as structural replacements (cosmetic reconstruction, for example), functional replacement (such as musculoskeletal tissues), and in wound healing (such as autologous skin grafts for burns).

Tissue engineering and regeneration involves the ex vivo production and/or in vivo regeneration of replacement organs and tissues using cell precursors and natural products and/or synthetic biomaterials as scaffolds to support the repair, regeneration, remodeling and eventual structural and/or functional replacement of injured, diseased and pathological tissues.

Key Ingredients

The key ingredients of tissue engineering and regeneration are cell precursors, natural products and synthetic biomaterials. Cell precursors can be autologous (from a skin biopsy) or heterologous (from a tissue bank of keratinocytes [epithelial]) cells derived from healthy or pathological tissue; mesenchymal stem cells; embryonic stem cells; and genetically manipulated cells. The following diagram shows how embryonic stem cells may be used.



Genetically manipulated cells include cells which are permanently or transiently transfected with a gene to either maintain a cellular phenotype or produce an important gene product that is needed for the tissue engineering construct. Often, large scale bioreactors are employed to culture the target cell outside of the body (ex vivo). This, however, can often introduce problems of cell dedifferentiation that needs to be controlled or regulated in order to produce large quantities of the desired product.

Natural Products

Natural products include autologous or heterologous fibrinogen, purified collagen (type I), coral, hyaluronan and human placenta. Fibrinogen, a natural circulating protein, can be used as a biological glue to entrap cells or facilitate adhesion of engineered implants into a wound or defect

site. Fibrinogen is not immunogenic and it is readily biodegradable. It can be easily isolated from the blood of the patient.

Following injury, insoluble Fibrin (the major insoluble part of a blood clot) is formed from fibrinogen. In blood clotting, fibrin clots are strengthened naturally by the crosslinking of the fibrin fibrils by a transglutaminase (factor XIII). Therefore, purified transglutaminases are also used in tissue engineering procedures to crosslink natural and synthetic scaffolds as well as hold implants in defects during repair.

Synthetics

Synthetic biomaterials include polylactic acid and polyglycolic acid, which are degradable; and carbon fibres and hydroxyapatite. Synthetic biomaterials have the advantage that they can be moulded into a variety of shapes and sizes that can later be customised into what is needed for a specific tissue engineering replacement. Synthetic/natural composites include collagen sponges, crosslinked hyaluronic acid (HA) and crosslinked HA with bioactive peptides to facilitate cellular migration and attachment. For example, autologous cartilage chondrocytes have been culture expanded and infiltrated into a collagen sponge *ex vivo* prior to its implantation into a large cartilage defect in the Fetlock joint of a race horse. The same types of treatment could be used to treat the pathology of knee joint issues in humans.

Natural Scaffolds

Natural products such as collagen can be engineered into a variety of different biodegradable scaffolds.

Functions of Natural Products and Biomaterials

The function of natural products and biomaterials is to provide a biodegradable scaffold (structural support for cells and ECM production); and to provide a meshwork for cellular infiltration.

Knee Joint Pathology

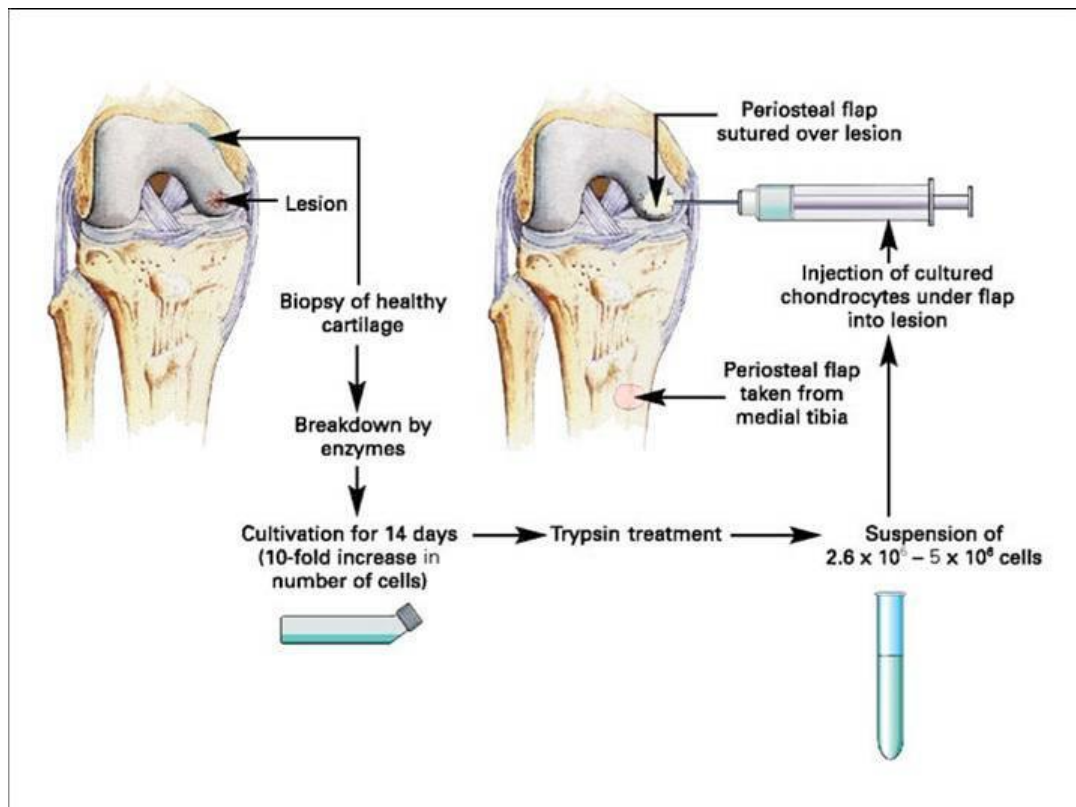
Tissue Engineering/Regeneration offers a means of treating joint injuries and defects prior to the need for joint replacement surgery. Conditions include anterior cruciate ligament injuries, collateral ligament injuries, meniscal cartilage tears and cartilage bruising (osteochondritis dissecans).

In meniscal repair, an allograft (dead tissue bank) or tissue engineered meniscal implant is sown (sutured) into the site of a partially removed meniscus at an injury site. Cells (chondrocytes and/or stem cells) from the adjacent uninjured tissue or nearby blood vessels migrate into and grow within the allograft or the *ex vivo* produced meniscal implant. After several months/years the implant is resorbed and remodelled by the cells thereby regenerating the meniscus and restoring joint function.

Osteochondrosis Dessicans (OCD)

OCD is bruising of the cartilage that often leads to the release of a fragment of cartilage and subchondral bone from the articular surface (forming a 'loose body'). This leads to joint inflammation, swelling and loss of function. The disease is more common in adolescents and young adults and the knee, ankle and elbow are most commonly affected. It is a common injury in professional cricketers & footballers. If left untreated, the cartilage does not usually repair.

Autologous Chondrocyte Transplantation (ACT) Autologous Chondrocyte Implantation (ACI) Procedure



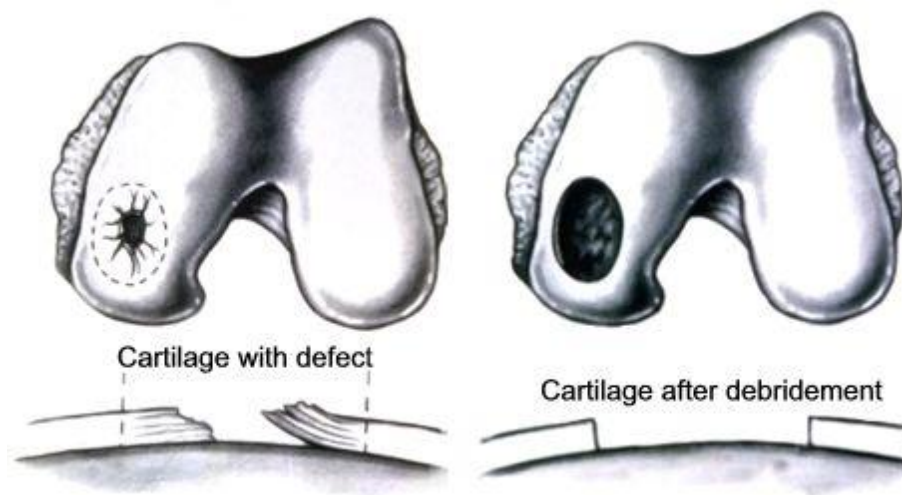
Tissue Engineering/Regeneration – Cartilage Repair (ACI/ACT)

Stage 1

First, an arthroscopic examination is performed to confirm OCD and any loose bodies within the joint space are removed. This is followed by harvesting of 'healthy' cartilage from the non-weightbearing areas of the injured joint. Cartilage pieces are used to isolate autologous chondrocytes that are culture expanded ex vivo. This takes about 3 to 4 weeks.

Stage 2

About 3-4 weeks later, the patient undergoes joint arthroplasty (open knee joint surgery). The injury site is debrided (cleaned).

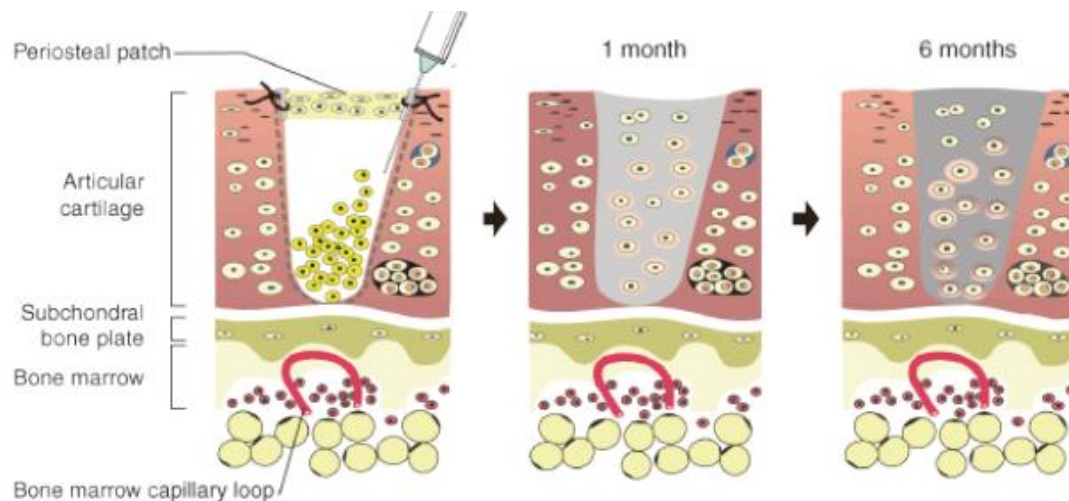


At the same time, periosteum (a tissue which covers the surface of all long bones) is harvested from the tibia and cut into a piece that covers or fits into the debrided hole in the cartilage surface. This is then sutured in place to the healthy cartilage, thereby covering the whole debrided cartilage defect. A solution of fibrinogen is then mixed with thrombin and used as a biocompatible “glue” to seal the edges of the periosteal covering that was sutured over the defect. The autologous chondrocytes (from ex vivo culture) are then injected under the sealed periosteal flap where they subsequently will divide and produce a repaired cartilage extracellular matrix; approx. 1 - 2 years. The knee joint is closed; for the next few days, the patient is given continuous passive motion of the knee then aided (crutches) ambulation and ‘normal’ weightbearing.

Key Components of Cartilage Repair

- Autologous chondrocytes from healthy cartilage for ex vivo culture expansion.
- Periosteum: a means to cover the defect and also a source of potential stem cells and growth factors to maintain the cell phenotype and promote repair.
- Fibrinogen and thrombin (usually isolated from the patient’s own serum) to be used to seal the periosteal piece over the defect.

Summary of ACI/ACT Procedures



Second & Third Generation ACI/ACT

Second and third generation ACI/ACT makes use of a collagen membrane rather than a periosteum flap, use of cell-seeded collagen membranes (MACI) and use of 3D scaffolds (such as HA), seeded with cultured autologous chondrocytes.

Learning Objectives and Review Questions

1. What are some of the key ingredients used in Tissue Engineering procedures?
2. What types of precursor cells are used?
3. What types of natural products are used?
4. What synthetic biomaterials are used?
5. What are the functions of the natural product and biomaterials used in Tissue Engineering?
6. How is Tissue Engineering used in Meniscus repair?
7. How is Tissue Engineering used in Cartilage repair?