TISSUE REPAIR

This pdf was developed by
Stephan Grundy
for ReviewPathology.com

Note on References

See box to the right for reference notations.

PubMed references usually link to abstracts, which provide useful information. But most abstracts link to a free, full-text article for in-depth reading.

Image references usually link to a whole page of images. The images themselves may be useful, but care must be taken to ensure that they relate to the topic of the text. Images are most valuable as a gateway to publications related to the topic. Clicking on the image will take you to the publication. However, to get the full publication it sometimes may be necessary to copy the url and transfer it to your browser.

- **Reference notations: when references are listed, the following suffixes indicate:**
- **pm = PubMed**
- **w = Wikipedia**
- **i = Images (usually Google images)**
- **rg = ResearchGate**
- **yt = YouTube**
- **sd = Science direct**
- **ow = Other website**
- **Selected text = PubMed, Wikipedia, or images**

What are the two major responses to tissue injury?

- Regeneration of tissues
- Scar formation

What are the mechanisms of cell and tissue regeneration?

- Cell proliferation (driven by growth factors)
- Development of mature cells from stem cells

What types of cells proliferate during tissue regeneration?

- Remnants of the injured tissue
- New vascular endothelial cells --> new vessels
- New fibroblasts --> scar tissue

What regulates cell proliferation?

- The cell cycle
- Growth factors
- Interaction of cells with extracellular matrix

What does scar formation achieve?

Puts a protective “patch” over injured tissue.

Phases of Tissue Repair

What are the phases of tissue repair?

The phases of tissue repair (1ow, 2i, 3yt)

- Haemostatic phase
- Inflammatory phase
- Proliferation phase
- Re-epithelialization phase
- Maturation or remodeling phase

What is the inflammatory phase (preceding initiation of tissue repair)?

- The inflammatory phase is characterized by the dilation and increased permeability of local blood vessels.
- White blood cells, primarily neutrophils and macrophages, scavenge cells and tissue components that are too injured to recover.
- Growth factors, enzymes, and nutrients permeate the region to begin the active healing process.
- The inflammatory phase is clinically described as “rubor, tumor, calor, dolor, and functio laesa” – redness, swelling, heat, pain, and loss of function.
- The normal duration for the inflammatory phase is ca. 3-6 days.

What is the haemostatic phase?

- The initial response to tissue damage is platelet activation (1i, 2i, 3ow) in order to create a temporary thrombotic plug in the vasculature at the site of injury.
- Platelet activation occurs concurrently with the initiation of the clotting cascade (1w, 2i, 3ow) The clotting cascade is slower to complete, but the formation of fibrin significantly strengthens the platelet plug.

What is the proliferation phase of wound healing?

- In proliferation phase (1pm, 2rg, 3rg), which begins approximately 24 hours after the original injury and may last up to four weeks, the tissue either regenerates directly or repairs injury by means of connective tissue and extra-cellular matrix proliferation.
- Regeneration is seen in labile tissues such as skin, bone marrow, and gut, and some organ parenchyma, particularly the liver, which can fully regenerate up to 60% of its mass.
- Severe injury or injury to stable tissues requires fibroblast migration and proliferation. This creates granulation tissue, which should be visible within 3-5 days.

What is the histologic appearance of granulation tissue?

Granulation tissue (1pm, 2i, 3sd) exhibits:

- Multiple small, delicate capillaries (produced by angiogenesis) (1rg, 2rg) .
- Visible fibrous structure within a loose extra-cellular matrix.
- Inflammatory cells (1w) are still visible, but reduce in number as the healing process continues.

What is the maturation/remodeling phase?

- In this phase, collagen is synthesized by fibroblasts and is remodeled from type III collagen (reticular fibre network) to type 1 collagen (skin, tendons, bone, vasculature, scar tissue).
- Some of the fragile new capillaries degenerate and disappear, while others strengthen their walls to become permanent blood vessels.
- Vascular regression (1a-pm, 1b-pm, 2pm) continues progressively as the scar ages.

Ulceration

(see images for varieties of ulcers)

What is ulceration?

- Ulceration is a break in a bodily surface. It is the result of a delay or chronic failure of the healing process.
- Normal surface layers are lost (usually due to necrosis) and replaced with inflammatory tissue.
- Ulcers may either resolve after an acute period or persist and become chronic.
- Ulcers are often painful, sometimes debilitating and/or disfiguring, and present an increased risk of localized infection.

What is the most important division of ulcer types?

- The most important division of ulcer types is:

- Malignant – an ulcerated cancer (such as the "rodent ulcer" of basal cell carcinoma) or cancer arising from an original ulcer (e.g., malignant "degeneration" of a gastric ulcer) (1i, 2w).

- Benign – an inflammatory ulcer without neoplasia. Presents dangers of infection, loss of function, pain, and disfigurement.

What are the three main types of lower extremity ulcer?

- Venous (stasis) (1ow, 2ow)
- Arterial (ischaemic) (1w, 2, 3pm)
- Diabetic (1i)
- Mixed venous/arterial ulcers may also occur.

What are the characteristics of arterial ulcers?

The characteristics of arterial ulcers (1ow) are:

- **Deep (may even expose tendons).**
- **Dry base.**
- **Punched-out edges.**
- **Surrounding skin may be pale, cool, shiny, and hairless.**
- **Distal pulses often absent.**
- **Typically seen in the context of peripheral arterial disease.**
- **More painful than venous** ulcers**.**
- **Commonly located at toe-tips, over phalangeal heads, lateral malleolus, middle tibia, and trauma sites.**

What are the characteristics of venous ulcers?

Venous ulcers (1ow, 2i) are characterized by:

- **Wet base with exudates and/or sloughed material.**
- **Shallow with sloping edges.**
- **Surrounding skin may be oedaematous and/or discoloured with haemosiderin.**
- **Typically a feature of venous stasis/valvular dysfunction.**
- **Most commonly seen on the "gaiter" area (medial distal leg).**
- **Comprise 70-90% of leg ulcers.**

What are the types of diabetic foot ulcer?

- Neuropathic – secondary to peripheral neuropathy allowing repetitive stress injuries leading to bone remodeling (increasing stress at contact points), untended blisters or other injuries which, exacerbated by poor circulation and metabolic imbalances, are prone to ulcerate. Normally seen on pressure points (ball and heel of foot, or other chronic points of contact. Typically painless, surrounded by a thick ring of callus.
- Ischaemic – peripheral vascular disease is seen earlier and more severely in patients whose diabetes is not well controlled. May lead to dry gangrene and amputation or auto-amputation of digits (1i).
- Neuroischaemic – usually initiated by friction blisters around the margin of the foot.

What are other significant ulcer types?

- **Peptic ulcers (1pm, 2pm, 3rg) – gastric, duodenal, oesophageal. Most commonly caused by *Helicobacter pylori* (1pm, 2i). *H. pylori* presence is confirmed by carbon breath** testing or stool examination for foreign proteins, and treatment is by means **of "triple therapy" – a proton pump inhibitor, clarithromycin, and amoxicillin (or metronidazole for patients with penicillin allergies). GI bleeding, sometimes sudden and severe, is the most common complication; *H. pylori* also increases the risk of gastric cancer by 3-6x.**
- **Pressure or decubitus ulcers (1pm, 2i, 3ow) result from ongoing pressure on soft tissue/skin above bony spots. They are common among patients restricted to bed or with limited mobility (e.g., wheelchairs): such patients require attention from hospital staff to make sure their positions are shifted frequently. Impaired circulation and skin fragility also contribute to the likelihood of developing pressure ulcers.**

What are the main principles of treating skin ulcers?

Treatment of skin ulcers

DOMINO:

- **Debridement**
- **Oxygenation**
- **Moisture control (moisten dry ulcers, dry wet ones)**
- **Infection control (diabetic ulcers are particularly prone to infection)**
- **Nutrition (crucial to healing of any sort)**
- **Offloading (taking weight/pressure off the lesion site)**

Wound Healing

What is the basic difference between healing by primary and secondary intention?

- The two are a continuum of the same processes.
- Healing by primary intention results in no scarring.
- Healing by secondary intention results in scarring.

What processes are common to all wound healing?

- Angiogenesis: new blood vessel development
- Activation of fibroblasts
- Deposition of connective tissue (1i)
- Remodeling of connective tissue (1i)

What are key steps in angiogenesis?

Angiogenesis (1pm, 2i, 3i, 4i) involves:

- Vasodilatation (due to nitrous oxide)
- Opening up of basement membrane
- Transmigration of endothelial cells
- Proliferation of endothelial cells
- Remodeling of capillary tubes
- Recruitment of periendothelial cells
- Deposition of a new basement membrane

What factors drive angiogenesis?

Factors that drive angiogenesis (1pm, 2i, 3i)

- **Growth factors:**
  - **VGEFs, (esp. VGEF-A)**
  - **Fibroblast growth factors**
  - **Platelet derived growth factor**
  - **TGF-beta**
- **Notch signaling pathway**
- **Extracellular matrix (ECM) proteins**
- **Matrix metalloproteinases**

What are the steps in laying down of connective tissue in response to tissue injury?

- Migration and proliferation of fibroblasts into the site of injury
- Deposition of extracellular matrix proteins produced by fibroblasts

What is the critical cytokine for the synthesis and deposition of connective tissue proteins?

- Transforming growth factor beta (TGF-β)

What are the three classical types of wound healing?

Three types of wound healing

- Primary intention
- Secondary intention
- Tertiary (“delayed primary”) intention

What is healing by primary intention?

Wound healing by primary intention consists of:

- **New connective tissue and blood vessels forming on the surface of a wound.**
- **Components include:**
  - **Fibroblasts**
  - **Thin-walled capillaries**
  - **Inflammatory cells**
  - **Loose extracellular matrix**

More on healing by primary intention)(1i, 2i, 3i)?

- Healing by *primary intention* occurs with clean, narrow wounds, where the edges are neatly juxtaposed.
- One of the main purposes of suturing a wound is to allow and promote healing by first intention.
- The initial small cleft is filled by blood clot and debris immediately.
- 2-3 hours – early inflammation around wound edges seen; epithelial cells begin to migrate across the wound.
- 2-3 days – macrophages remove clot and debris. Blood vessels proliferate; fibroblastic activity is seen.
- 10-14 days – epithelial coverage complete; weak fibrous union of edges
- Weeks – improved fibrous union up to 70% of original strength.
- Months to years – collagen remodeling and devascularization.

What are keloids?

- Excessively large scars

- **Result from excessive production of extracellular matrix (1i, 2i, 3i)**

What is healing by secondary intention?

- Healing by *secondary intention* (1i, 2i) occurs in wounds where there has been a significant infection or loss of skin or tissue.
- The cavity fills with a blood/fibrin clot and acute inflammation starts at the living-tissue border.
- Mitosis of the surrounding epithelium pushes a sheet of epithelial cells between the clot and the living tissue. Capillary angiogenesis supplies macrophages, neutrophils, fibroblasts. Wound contracture begins.
- 1 week – clot/debris are sloughed off. Granulation tissue is visible at the base of the wound.
- 2 weeks+ - epithelial covering is complete; collagen remodeling and loss of newly formed capillaries continues.

What are the chief concerns with healing by secondary intention?

- Greatly increased risk of infection; the wound requires daily cleaning and re-dressing.
- Greatly increased likelihood of visible scarring and wound contracture.
- Significantly increased time to full healing.
- Often the increase in time and pain, and the more visible and potentially disfiguring nature of the wound, have a significant negative impact on patients who undergo healing by secondary intention.

What is healing by tertiary intention?

- Healing by tertiary intention (1i, *2i*, 3i), or "delayed primary intention", is a relatively new concept.
- Tertiary intention is when the wound must be left open for a period of time before it can be sutured.
- Indications for tertiary intention include: the need for drainage; wounds healing by secondary intention which encounter complications; poor circulation to the relevant area; high risk of infection (animal bites, lacerations with foreign bodies) which indicate leaving the wound open for a few days to be sure there is no infection before fully closing.

What is wound contracture?

- Wound contracture is caused by traction exerted at the edges of the healing wound by myofibroblasts in the granulation tissue.
- The initial function is beneficial (bringing the edges of an open wound together).
- However, if the myofibroblast population continues to proliferate, wound contracture may lead to cosmetic and/or functional difficulties.
- This is particularly the case in regards to burn scars.
- Z-plasty (1i) may be performed after healing to prevent a problematic degree of contracture (https://www.youtube.com/watch?v=wdseg3UvXrI).

What factors impair tissue repair?

- Mechanical (increased pressure or torsion)
- Poor perfusion (atherosclerosis, diabetes, varicose veins)
- Foreign bodies (external fragments or bone)
- Perforating lesions
- Infection
- Inflammation
- Diabetic ulcers
- Large lesions

Cellular Proliferation

What happens when healing tissues overproliferate?

- 1 – Hypertrophic granuloma. Usually secondary to a wound infection during the healing process, granulation tissue burgeons.
- 2 – Keloid scar formation. Significant overproliferation of collagen and connective tissue. Can occur in any ethnic group and overall affect ca. 10% of the population, but are more common (15 x higher occurrence) in people of African descent.

Both of these are unsightly and may cause the patient significant aesthetic distress; very large or badly situated keloids may hamper function; but both hypertrophic granuloma and keloids are benign.

Which cells proliferate during tissue repair?

- Cells of injured tissue attempt to regenerate structure.
- Vascular endothelial cells (1pm, 2i) proliferate to bring new vessels to the injured area and thus supply nutrients for repair.
- Fibroblasts proliferate to form fibrous scar tissue in defects non-repairable by regeneration.

What is cellular senescence?

- Cellular senescence (1pm, 2pm, 3i)(replicative senescence) is a phenomenon whereby normal cells fail to divide.
- Shortening of the chromosomal telomeres with each cell cycle seemingly promote cell senescence.
- Cellular senescence with aging also may impair wound healing.
- Replicative senescence may occur in one phase (G1 vs G2) of the cell cycle. (1i, 2rg).

How does the cell cycle work?

The cell cycle is regulated as follows:

- Non-proliferating cells are either quiescent in G_0 phase or held static in the G_1 phase via the suppression of cyclin-dependent kinases (CDKs).
- Growth factors terminate the suppression of CDKs, which interact with cyclin proteins to allow progression through the cell cycle and promote DNA replication.
- Checkpoint controls (1i) are the defensive mechanism by which damaged DNA is removed from replication and severely damaged cells are induced to begin apoptosis.

What are the different types of tissues in terms of proliferation capability?

- **Continuously dividing or *labile* tissues (haematopoetic bone cells, epithelia) – readily regenerate after injury.**
- ***Stable* tissues (most solid tissue parenchyma, endothelial cells, fibroblasts, smooth muscle cells). Can proliferate in response to damage, but only to a limited degree (except for the liver, which readily regenerates).**
- ***Permanent* tissues – neurons, myocardial cells. Little to no proliferative capability; injuries are generally replaced by scar tissue.**

What role do stem cells play in proliferation?

- Stem cells are cells that can differentiate into other types of cells or produce more of the same kind of cells . They are primarily found in labile tissues, and provide the means of constant cell replacement.
- Embryonic stem cells are pluripotent; that is, they can differentiate into multiple cell lineages.
- Adult, or tissue, stem cells are normally specific to the tissue where they are found.
- However, bone marrow stem cells can differentiate broadly (bone, cartilage, endothelium, muscle, or fat).
- Adult stem cells can also be induced to become pluripotent.

Who are Shinya Yamanaka and John Gurdon?

- Winners of the 2012 Nobel Prize for “the discovery that mature stem cells can be reprogrammed to become pluripotent”.
- They induced pluripotent stem cells to offer a wide range of possible therapies for the future: for instance, the ability to generate replacement cells or tissues from the patient’s own cells, thereby avoiding issues of graft rejection.

Growth Factors

What is the potential of growth factors for wound healing?

- In 1996, Greenhalgh speculated about the role of growth factors for wound healing. It was noted that:
- Growth factors have activities that could stimulate tissue repair.
- They attract cells into the wound, stimulate proliferation, and promote extracellular matrix deposition.
- However, to date, they have not proven to be a panacea for wound healing.
- But a better understanding the role of growth factors for wound healing still holds potential.

How do growth factors affect cells?

- Extracellular signaling (ligand binding to a receptor on the cell to initiate signaling pathway within the cell).
- Types of cellular signaling include:
  1. Autocrine – largely acts back on the same cell that secretes a substance. Notably seen in the immune system.
  2. Across gap junctions (Juxtacrine signaling) – cell signals a neighboring cell to which it is attached by a gap junction.
  3. Paracrine – largely affects cells in the immediate vicinity of the secretory cell, e.g., inflammatory cell recruitment to a site of damage.
  4. Endocrine – hormones released into the bloodstream to target both local and distant cells.

What is epidermal growth factor (EGF)?

- Epidermal growth factor (EGF) (1i, 2rg, 3rg) is a secreted peptide which binds to EGFR (EGF-receptor) on the cell surface membrane to initiate an intracellular pathway which promotes mass increase, DNA synthesis, and cellular proliferation.
- EGF is produced by activated macrophages, keratinocytes, and salivary glands, among other cellular sources. It is found in saliva, milk, urine, and plasma.
- EGF promotes mitogenesis in keratinocytes and fibroblasts. It is crucial to the healing process, including granulation tissue formation (1w) and scar formation.
- Recombinant EGF has been studied therapeutically for non-healing cutaneous wounds such as diabetic foot ulcers.

What is transforming growth factor-α?

- Transforming growth factor-α (TGF-α) (1i, 2i, 3i) is a growth factor which stimulates epithelial cell and hepatocyte replication and may contribute to angiogenesis.
- Produced by activated macrophages, keratinocytes, T-cells, and brain cells.
- Functions as a ligand for the EGF receptor, initiating the pathway which leads to cell DNA synthesis and replication.
- Involved in both wound healing and tumorogenesis.

What is transforming growth factor β?

- Transforming growth factor beta (TGF-β) (1i, 2i, 3i) is produced by platelets, T-cells, macrophages, endothelial cells, keratinocytes, smooth muscle cells, and fibroblasts.
- It controls cell growth, proliferation, differentiation, and apoptosis.
- It is chemotactic for macrophages, lymphocytes, neutrophils, keratinocytes, smooth muscle cells, and fibroblasts.
- It inhibits activation of T_H and cytotoxic T-cells; suppresses proliferation of monocytes and macrophages; but can also chemotactically direct immune system cells to a local site and stimulate the production of cytokines.
- It stimulates fibroblast proliferation, angiogenesis, and tissue inhibitor of matrix metalloproteinases (1pm, 2pm).

What is vascular endothelial cell growth factor (VEGF)?

- Vascular endothelial cell growth factor (VEGF) (1pm, 2w, 3i, 4i) is a growth factor that increases existing blood vessel permeability, promotes endothelial cell migration and mitosis, and provides the means for angiogenesis to oxygenate damaged or growing tissues. It has both positive and negative effects, depending on the circumstances of production.
- The VEGF family has several members, of which VEGF-A is the most studied.
- VEGF-A production is induced when a hypoxic cell produces HIF (hypoxia-inducible factor), promoting angiogenesis.
- VEGF is produced by mesenchymal cells.

What role does VEGF-A play in disease?

- VEGF-A (1w, 2i, 3i) is implicated in many disease pathologies. These include:
- Diabetic retinopathy and wet age-related macular degeneration (promotes angiogenesis in the retina).
- Rheumatoid arthritis (angiogenesis and increased vascular permeability in the joints lead to joint swelling).
- In some forms of cancer, VEGF-A promote cell migration, tumour proliferation, and metastasis; angiogenesis permits solid tumours to continue growing without being restricted by lack of blood supply).
- VEGF-A can cause direct glomerular hypertrophy, leading to proteinuria.

What is hepatocytic growth factor (HGF)?

- Hepatocytic growth factor (HGF) (1w, 2i, 3i) controls cell growth, motility, and morphogenesis.
- It is a paracrine factor produced by mesenchymal tissue.
- It stimulates proliferation of hepatic, epithelial, endothelial, haematopoetic, and T-cells.
- It upregulates T-cell chemotaxis into cardiac tissue.
- HGF is an important factor in cancer development and metastasis, stimulating cancer cells to divide from the main tumour and increases their motility.

What is platelet-derived growth factor (PDGF)?

- Platelet-derived growth factor (PDGF) (1w, 2pm) is produced and stored largely by platelets, but it is also produced by macrophages, endothelial cells, keratinocytes, and smooth muscle cells.
- It stimulates angiogenesis and mesenchymal cell proliferation and migration.
- It is required for fibroblast division; and it speeds fibroblast proliferation and shortens time to healing.
- Recombinant PDGF is used to promote the healing of chronic ulcers and to stimulate bone regeneration/repair.

What are fibroblast growth factors (FGFs)?

- Fibroblast growth factors (FGFs) are a family of growth factors (22 identified in humans thus far).
- Produced by a variety of tissues, macrophages, mast cells, T-cells, endothelial cells, and fibroblasts.
- FGFs promote chemotaxis in fibroblasts, proliferation in fibroblasts and keratinocytes, keratinocyte migration, angiogenesis, wound contraction, and matrix deposition.
- FGFs 1 and 2 are more powerfully angiogenic than VEGF or EGF.
- Considered “promiscuous” or “pluripotent” growth factors due to their effects on a range of cell types.

What is keratinocyte growth factor (KGF)?

- Keratinocyte growth factor (KGF) (1pm, 2pm) is a growth factor produced by fibroblasts.
- It stimulates differentiation, proliferation, and migration in keratinocytes.
- This allows keratinocytes to fill in gaps in the epidermis (e.g., wounds) -.

Extracellular Matrix (ECM)

What is the extracellular matrix (ECM)?

- The extracellular matrix (ECM) (1w, 2pm, 3rg, 4rg) binds and supports cellular structures.
- It has various compositions for various tissues:
- Epithelial cells rest on the basal membrane, as seen, for instance, in glomeruli.
- Interstitial matrix supports, hydrates, and buffers in the intracellular spaces.
- Collagen fibres and bone minerals serve as the ECM for bone tissue.
- Plasma takes the extracellular matrix's role in blood.
- The ECM plays a crucial part in tissue repair and regeneration.

What are the main components* of the extracellular matrix?

- Fibrous proteins (collagens, elastin, laminin).
- Glycosaminoglycogens (chondroitin sulfate, keratan sulfate, heparan sulfate, heparin, dermatan sulfate, hyaluronin).
- Fibronectin.

* From Wikipedia

What is the function of collagen?

Collagen (1i, 2i, 3sd, 4w) is the most crucial supporting fibrous protein.

It is crucial to aiding wound healing by means of guiding fibroblasts to migrate, attracting fibrogenic cells, providing a nucleating structure for fibrillar structures, and (together with platelets) forming wound plugs (1i, 2i).

Collagen forms skin, vascular layers, the organic portion of bone, tendons, and organs (type I collagen [1pm]); cartilage (type II collagen [1pm]); soft tissue/organ reticular fibre networks and granulation tissue (type III collagen [1pm, 2i]); basil lamina (type IV collagen [1pm, 2nejm]); cell surfaces, hair, and much of the placenta (type V collagen [1pm]).

28 types of collagen have been identified, but type I predominates at ca. 90%.

What are the main clinical uses and diseases of collagen?

- Collagen is used as scaffolding and dressings to promote regeneration and wound closure (1pm)., i.e., in bone grafts and for large-area wounds, especially burns; and as a means of delivering topical medications (1pm).
- Notable collagen-related genetic diseases are Ehlers-Danlos syndrome and osteogenesis imperfecta.
- Scurvy (1w) is caused by a lack of vitamin C (1w), which is essential to collagen synthesis. It presents with poor wound healing, perifollicular papules which extend to purpura (1i), the opening of old scar tissues, bleeding gums, and lost teeth.

What is elastin?

- Elastin is a form of connective tissue that allows for both stretching and re-contraction.
- All tissues in which the ability to stretch and contract is crucial (lungs, arteries, skin, bladder) require elastin.
- Defects in elastin metabolism lead to conditions such as:
- α_1-antitrypsin deficiency (1pm) asthma (elastase is not controlled and therefore breaks down elastin in lungs, causing emphysema)
- Marfan syndrome (1yt, 2pm)(tall, thin, flexible phenotype; increased risk of aortic aneurysm and mitral valve prolapse)

What is laminin?

- Laminins are high molecular-weight, trimeric proteins which form crucial elements of the lamina of basal membranes (1i).
- They usually appear in association with type IV collagen and bind to cell membranes.
- Laminins play a major role in cell migration, differentiation, shape, and survival.
- Defects in laminin production can lead to nephrotic syndrome, Herlitz-type junctional epidermolysis bullosa (lack of laminin 332), and muscular dystrophy.

What are glycosaminoglycogens (GAGs)?

- Glycosaminoglycogens (GAGs) (1i) are highly polar, hydrophilic, and incompressible chains of unbranched polysaccharides with a repeating disaccharide unit.
- The combination of water attraction and incompressibility allows GAGs to form a “spongelike” matrix that serves as a shock absorber.
- GAGs form about 10% of the extra-cellular matrix (1i).

What are the main groups of GAGs?

- Hyaluronan
- Chondroitin sulfate and dermatan sulfate
- Heparan sulfate and heparin
- Keratin sulfate

What is hyaluronan (hyaluronic acid)?

- Hyaluronan (hyaluronic acid) (1i) is the largest GAG (ca. 25,000 disaccharide units).
- Simplest GAG (non-sulfated; not including complex arrangements of various disaccharides; does not link to proteins to form proteoglycans.
- Produced by an enzyme complex in cell membranes.
- Major component of synovial fluid, auricular cartilage, and skin.
- Increases viscosity; serves as a lubricant and shock absorber.
- Dysregulation of hyaluronan synthesis may play a role in progression of breast cancer.

What are chondroitin sulfate and dermatan sulfate?

- Chondroitin sulfate (1i) and dermatan sulfate (1i) are:
  - sulfated, complex (multiple types of disaccharide unit; always found complexed with proteins to form proteoglycans), small (ca. 300 disaccharide units), produced within the cell and released by exocytosis.
  - N-acetylgalactosamine alternates with glucuronic acid (1i).
  - Chondroitin sulfate is converted to dermatan sulfate by epimerization of any glucuronic acid residues to iduronic acid (via glucuronyl to iduronyl epimerase).

What are the functions of chondroitin sulfate and dermatan sulfate?

- Inhibit coagulation
- Contribute to prenatal brain development
- Influential in liver development and regeneration
- Significant contributors to wound repair (notably dermatan sulfate)
- Deficiency in dermatan sulfate –> Ehler-Danlos syndrome (EDS), progeroid EDS.
- Deficiency in chondroitin sulfate synthesis –> Temtamy preaxial brachydactyly syndrome. This syndrome is characterized by brachydactyly, dysmorphic facies, sensorineural hearing loss, dental abnormalities, hyperphalangism (1i), growth retardation, delays in motor and cognitive development.

What is heparan sulfate?

- Heparan sulfate (1i) is a GAG produced by almost all cell types and forms part of the ECM.
- Heparan sulfate binds to core proteins to form proteoglycans (1i), which are released into the ECM, stored in granules for later secretion, or attached to the plasma membrane.
- Heparan sulfate has anticoagulant properties, but they are much weaker than those of heparin.
- Heparan sulfate binds and protects protein factors such as growth factors and cytokines, promoting their activity in healing tissue.

What is heparin?

- Heparin (1i, 2i) is an anticoagulant produced by mast cells and basophils.
- It is usually released as part of the inflammatory process, and is commonly used as an anti-thrombotic prophylactic for bed-bound patients.
- Heparin binds to and activates antithrombin III, which in turn inactivates thrombin and coagulation factor Xa.
- Low molecular weight heparin (LMWH), as contrasted to unfractionated heparin, lacks the density to bind thrombin, and therefore affects only Xa.
- Heparin is degraded by heparinases, with a half-life of 1-2 hours after infusion (unfractionated) or four times that for LMWH.
- Heparin is currently being investigated for its action in a number of disease states: autoimmune conditions, cancer, potential nano-carrier for drug delivery, adult respiratory distress syndrome, and transplant rejection.

What are the dangers of pharmaceutical heparin?

- Unfractionated heparin – risk of osteoporosis and heparin-induced thrombocytopenia (HIT). Overdoses may be fatal.
- These risks are reduced with LMWH, and further reduced with heparin analogues such as fondaparinux (Xa selective, mediates anticoagulation indirectly via interaction with antithrombin III).
- Fondaparinux can be used safely in patients with established HIT, but is cleared renally, so is not advised for patients with renal dysfunction.

What is keratan sulfate?

- Keratan sulfate (1i) describes a group of sulfated GAGs.
- Keratan sulfate is found most copiously in the corneas (ca. 10x the level in cartilage, 2-4x the level in other tissues) and in bone and cartilage.
- Keratan sulfate has functional roles in cellular recognition of protein ligands, axonal guidance, cell motility, and in embryo implantation.
- KS levels are consistently raised in patients with mucopolysaccharidoses and lipidoses.

Remodeling of Connective Tissue

What is achieved by re-modeling of the connective tissue and a scar?

- Increasing the strength of connective tissue
- Contracting the scar

What are the steps in connective tissue remodeling?

- More collagen cross-linking
- Increased size increased collagen fibers
- Change type III collagen to stronger type I collagen.
- Later shrinkage of the scar (due to collagen degradation effected by a family of *matrix metalloproteinases* (MMPs) (1w).

What conditions result from elevated levels of GAGs? (see section on individual genetic diseases for more details on mucopolysaccharidoses [MPS])

Hurler syndrome (1w, 2i) – alpha-L-iduronase deficiency causes MPS I characterized by elevated levels of heparin and dermatin sulfate. Autosomal recessive disorder with features of abnormal facies, mental impairment, corneal opacity; zebra bodies seen on microscopic examination of lysosomes (zebra bodies are also seen in lysosomal storage disorders such as Tay-Sachs and Fabry's disease). Incidence is raised in certain ethnic/cultural groups with traditions of consanguineous marriage; among Irish Travellers, the incidence is 1:371 with a 1:10 carrier rate, as contrasted to the normal incidence ratio of <1:100,000.

Scheie's syndrome – MPS with less severe form of Hurler's, without mental impairment and with a normal lifespan, but with corneal clouding and facial dysmorphia. Both Hurler's and Scheie's are classed as mucopolysaccharidosis I and Scheie's is MPS 1S.

Further mucopolysaccharidoses

- Hunter syndrome (MPS II) (1w, 2i). Deficiency of iduronate sulfatase; dermatan sulfate and heparin sulfate are elevated. X-linked recessive; no corneal clouding or mental impairment; joint stiffness and bone abnormalities common.
- Sanfilippo syndrome (MPS III) (1w, 2i). Multiple enzyme deficiencies; heparan sulfate is elevated. Severe nervous system deterioration, shortened lifespan, hirsuitism, hepatosplenomegaly, facial abnormalities.
- Morquio syndrome (MPS IV) (1w, 2i). Accumulation of keratan sulfate. Abnormal heart and skeletal development (especially spinal), impaired growth, corneal clouding; may have a shortened lifespan.

Further Mucopolysaccharidoses

- Maroteaux-Lamy syndrome (1w, 2i) MPS VI, autosomal recessive deficiency in arylsulfatase B causing buildup of dermatan sulfate. Normal mental development; spinal and cardiac abnormalities; restricted joint movement; coarse facies.
- Sly syndrome. (1w, 2i) MPS VII, autosomal recessive beta-glucuronidase deficiency. Buildup of dermatan sulfate, chondroitin sulfate, heparan sulfate. Moderate mental impairment and motor retardation, spinal deformity, coarse facies, repeated pulmonary infections, clouded corneas and iris coloboma, hepatomegaly, splenomegaly.