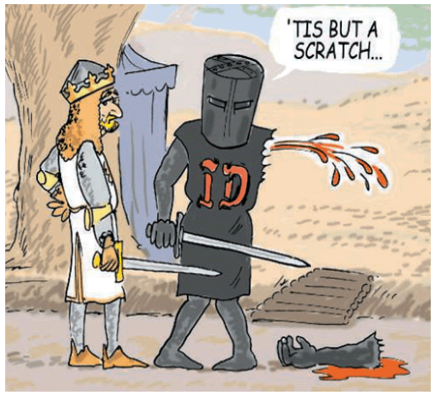
Tissue response to injury – wound healing

Acute inflammation
Regeneration vs. reparation
Scar formation
Remodelation

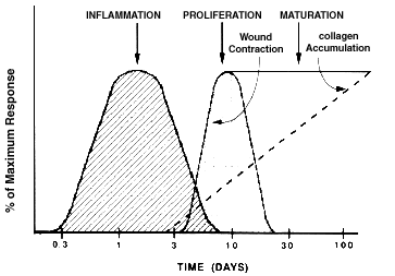
Types of wounds / damage

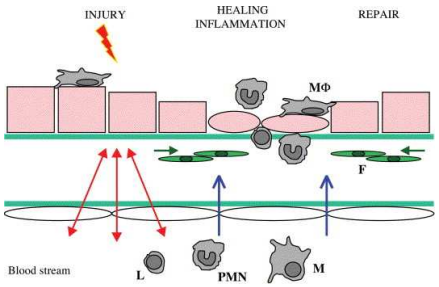
- traumatic
  - mechanical
    - laceration, incision, abrasion, ...
  - chemical
    - coagulation, burns, ...
  - physical
    - burns, frost-bites, ...
- ischemic necrosis
- biological
  - invasion of microorganisms with tissue-destructive properties
    - phlegmone, gangrene



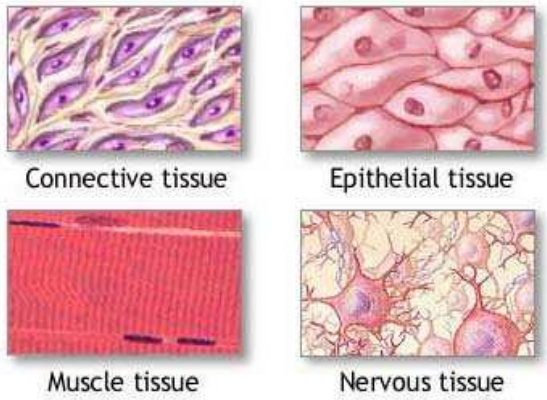
Overview of tissue response to injury

- healing is a sequential process leading to the resurfacing, reconstitution and restoration of the tensile properties of the tissue
- series of events
  - (1) haemostasis
    - plug & clot formation
    - soon followed by fibrinolysis to limit the extent clotting
  - (2) acute inflammation
    - to kill event. invading microoorganisms
    - to remove the necrotic tissue and cell debris
  - (3) followed by
    - (a) complete resolution (= regeneration)
    - (b) healing by repair
      - initially epithelisation, fibroplasia, angiogenesis
      - followed by maturation of the scar
    - (c) chronic inflammation





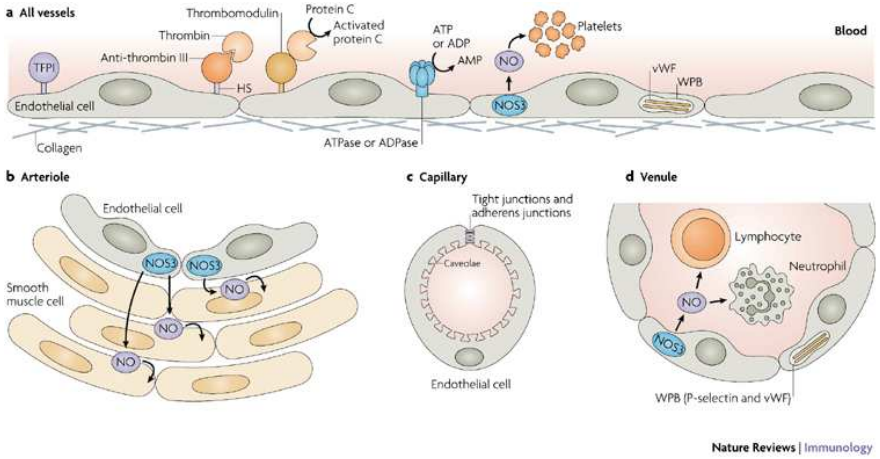
Four types of tissue



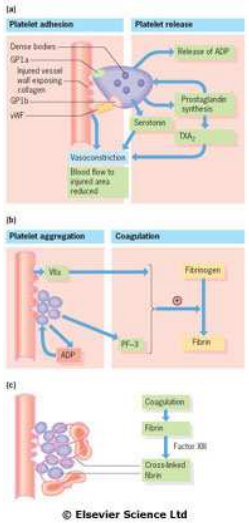
Endothelium: physiological role

- endothelial cells (ECs) normally **inhibit coagulation of the blood**
- tissue factor pathway inhibitors (TFPIs) prevent the initiation of coagulation by blocking the actions of the factor-VIIa–tissue-factor complex
- anti-coagulant heparan sulphate proteoglycans (HS) bind anti-thrombin III to be capable of inhibiting any thrombin molecules generated by the coagulation cascade
- thrombomodulin binds thrombin and converts its substrate specificity from cleavage of fibrinogen (the key step in forming a blood clot) to cleavage and activation of protein C
  - activated protein C is an enzyme that destroys certain clotting factors and inhibits coagulation
- key processes to prevent platelet activation (and therefore coagulation) include inactivation of thrombin, conversion of ATP to inert AMP through the action of ATPases and ADPases, and blocking the physical interaction between platelets and collagen, which can activate platelets
- sequestration of von Willebrand factor (vWF), a protein that strengthens the interaction of platelets with the basement membrane, by keeping it within their storage granules, known as Weibel–Palade bodies (WPB)
- nitric oxide (NO), generated by nitric-oxide synthase further inhibits platelet activation
- arterial endothelial cells have a major role in regulating blood flow by controlling the tone of smooth muscle cells in the medial layer of the vessel wall
- capillary endothelial cells are the principal regulators of transendothelial extravasation of plasma proteins (tight junctions and adherens junctions)
- venular endothelial cells form the principal site of leukocyte trafficking from the blood into the tissues

Endothelium

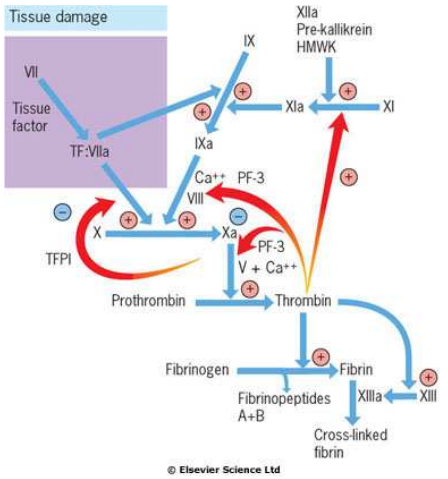


(1) Haemostasis: platelet plug formation & vasoconstriction



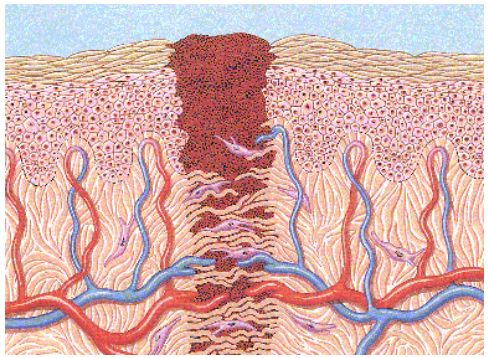
- protection against excessive bleeding = aggregation of platelets results in the formation of the **primary platelet plug**
- endothelial cells retract to expose the subendothelial collagen surfaces
- platelets attach to these surfaces
- aggregation and attachment to exposed collagen surfaces activates the platelets
- activation enables platelets to degranulate and release chemotactic and **growth factors**, such as platelet-derived growth factor (PDGF), proteases, and vasoactive agents (eg. serotonin, histamine)
- adherence to exposed collagen surfaces and to other platelets occurs through adhesive glycoproteins: fibrinogen, fibronectin, thrombospondin, and von Willebrand factor

(1) Haemostasis: clotting cascade



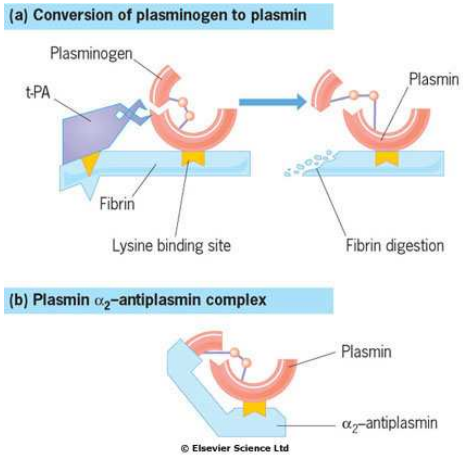
- coagulation cascade occurs by 2 different pathways
  - intrinsic pathway begins with the activation of factor XII (Hageman factor), when blood is exposed to intravascular subendothelial surfaces
  - extrinsic pathway occurs through the activation of tissue factor found in extravascular cells in the presence of factors VII and VIIa
- the result of platelet aggregation and the coagulation cascade is **clot formation**
- clot formation has to be limited in duration and to the site of injury
- both pathways proceed to the activation of thrombin, which converts fibrinogen to fibrin
- in addition to activation of fibrin, thrombin facilitates migration of inflammatory cells to the site of injury by increasing vascular permeability
  - by this mechanism, **factors and cells necessary to healing flow from the intravascular space and into the extravascular space**

Blood clot



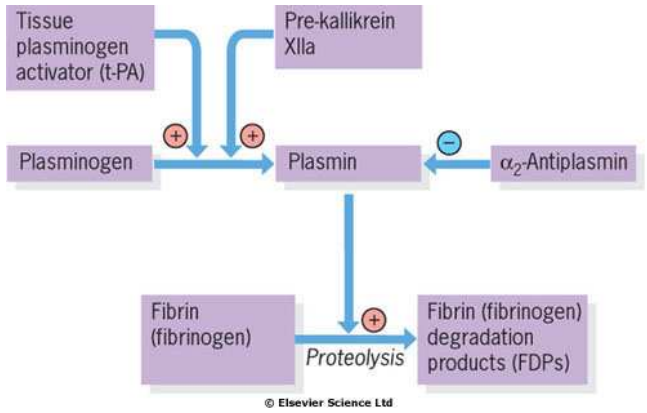
- **fibrin is essential to wound healing and is the primary component of the wound matrix** into which inflammatory cells, platelets, and plasma proteins migrate
  - removal of the fibrin matrix impedes wound healing

(1) Haemostasis vs. fibrinolysis



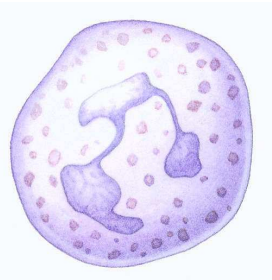
- clot formation dissipates as its stimuli dissipate
  - clot formation is limited to the site of injury because uninjured nearby endothelial cells produce prostacyclin, an inhibitor of platelet aggregation
- extent of coagulation is regulated by the action of:
  - fibrinolytic system
    - plasminogen is converted to plasmin, a potent enzyme dissolving blood clot
  - anticoagulant system
    - in the uninjured areas, antithrombin III binds vitamin K-dependent coagulation factors
    - protein C binds factors of the coagulation cascade, namely, factors V and VII

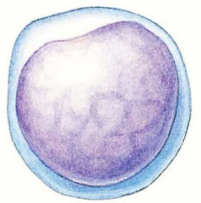
Fibrinolysis



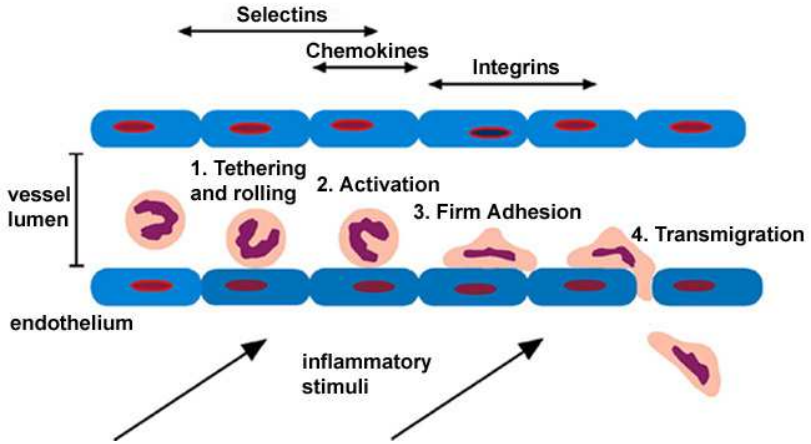
(2) Acute inflammation

- initial response to tissue damage
- relatively non-specific
  - excess of immune system to the damaged area
    - change of endothelial permeability (exudate)
    - adhesion and extravasation of immune cells
    - selectins, adhesive molecules (VCAM, ICAM, …)
    - chemotaxis
  - elimination of dead tissue
    - proteolysis (lysozomal enzymes), phagocytosis, ROS
  - protection against infection
    - initially PMNs (phagocytosis) – dye in site (= pus)
    - later monocytes/macrophages (phagocytosis, cytokines, initiation of tissue repair)
      - cytokines – completion of inflammation
      - growth factors – tissue repair
  - specific immune system (lymphocytes) not always necessary
    - viral infections
    - chronic inflammation

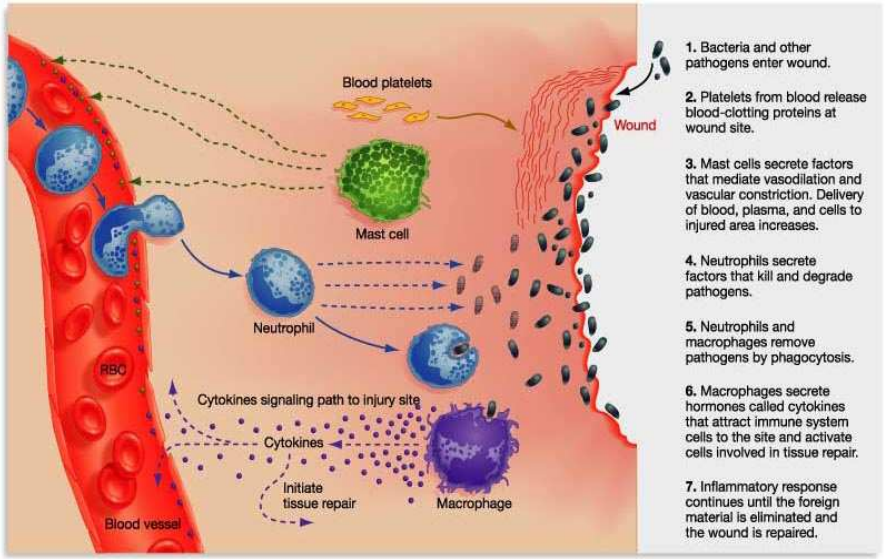




Activated endothelium

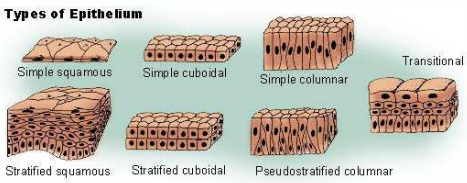


(2) Acute inflammation



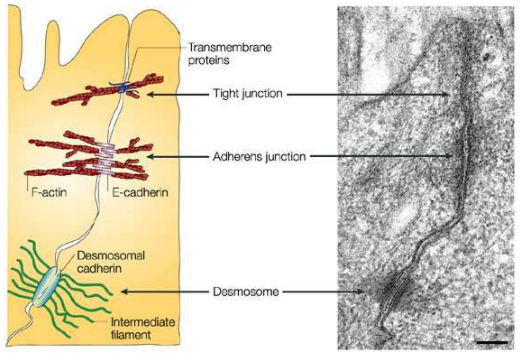
(3a) Complete resolution (= regeneration)

- the best possible outcome = restoration of normal structure and function without scarring
- occurs when damage to supporting stroma is minimal and mainly epithelial defect is present
- factors favouring regeneration
  - tissue has regenerative capacity
  - fast destruction of agent
  - fast removal of debris
  - good drainage
- regenerative potential of cells
  - labile cells
    - high turnover rate
    - high regenerative capacity
    - squamous, glandular, GIT epithelia, bone marrow
  - stable cells
    - low turnover rate
    - proliferative capacity can be increased when necessary
    - liver, kidney, fibroblasts, osteoblasts, endothelial cells, glia
  - permanent cells
    - cannot divide = cannot regenerate
    - heal with scar tissue
    - neurons, muscle cells

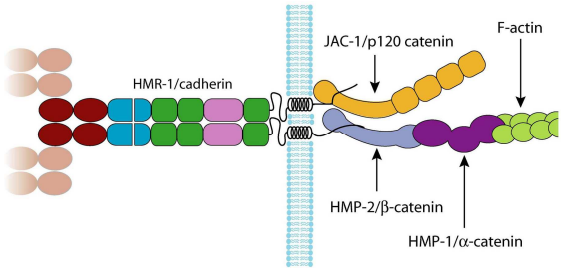


Intercellular junctions

- Tight junctions
  - transmembrane proteins that link to the actin cytoskeleton and prevent the leakage of small molecules through intercellular spaces
- Adherens junctions
  - homophilic interactions between E-cadherin molecules connected to the actin network through catenins
  - function to coordinate the actin cytoskeleton across an epithelial sheet
- Desmosomes
  - desmosomal cadherins linked to intermediate filaments
  - integrate the intermediate-filament network across the epithelial sheet
- Gap junctions
  - directly connects the cytoplasm of two cells, which allows various molecules and ions to pass freely between cells

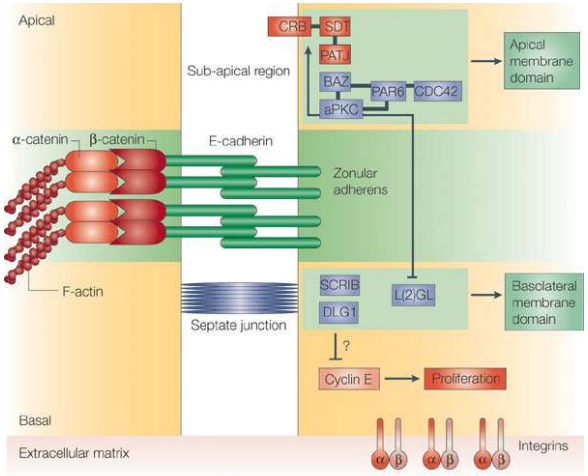


Cell-cell connection: E-cadherin

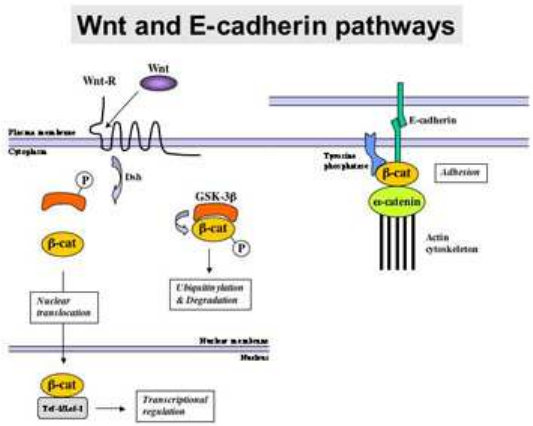


- class of type-1 trans-membrane protein, Ca+-dependent
- various types
  - E-cadherins in epithelial tissue
  - N-cadherins in neurons
  - P-cadherins in the placenta
- E-cadherin:
  - 5 cadherin repeats (EC1 ~ EC5) in the extracellular domain
  - one transmembrane domain
  - intracellular domain that binds b-and a-catenins and thus actin cytoskeleton
  - expressed in epithelial tissues, where it is constantly regenerated with a 5-hour half-life on the cell surface
  - loss of E-cadherin function or expression has been implicated in cancer progression and metastasis
    - E-cadherin down-regulation decreases the strength of cellular adhesion within a tissue, resulting in an increase in cellular motility

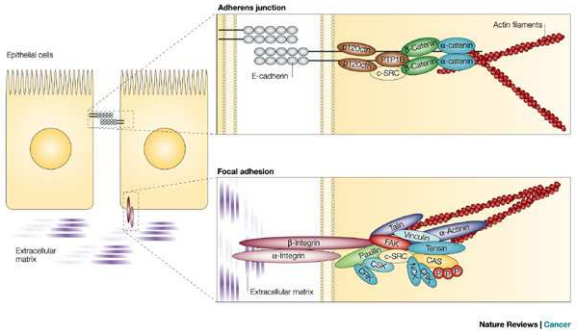
Principle of "contact inhibition"

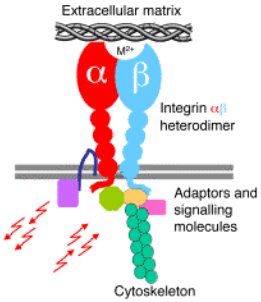


Loss of E-cadherin junction is a signal to proliferate

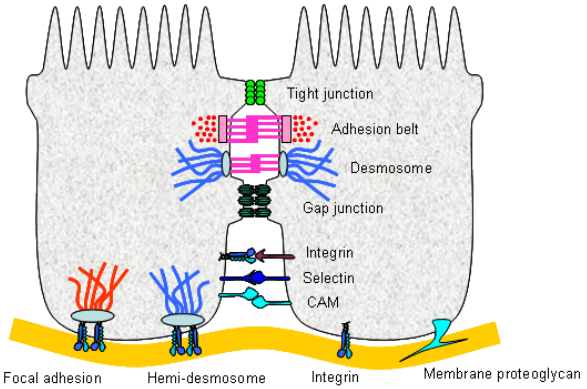


Cell-ECM connection: integrins



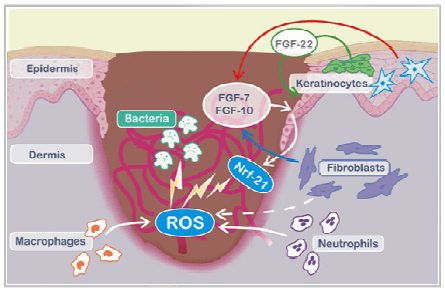


Summary of cell connections/adhesions



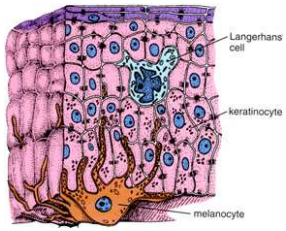
(3b) Tissue repair (= reparation)

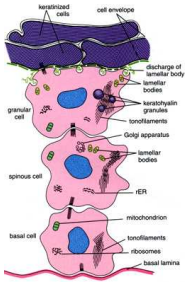
- type of healing occurring after substantial damage to the tissue stroma
- leads to the formation of scar
- sequence of processes
  - proliferation phase
    - epithelisation
    - angiogenesis
    - fibrotisation
  - subsequently, granulation tissue forms and the wound begins to contract
  - maturation phase
    - collagen forms tight cross-links to other collagen and with protein molecules, increasing the tensile strength of the scar



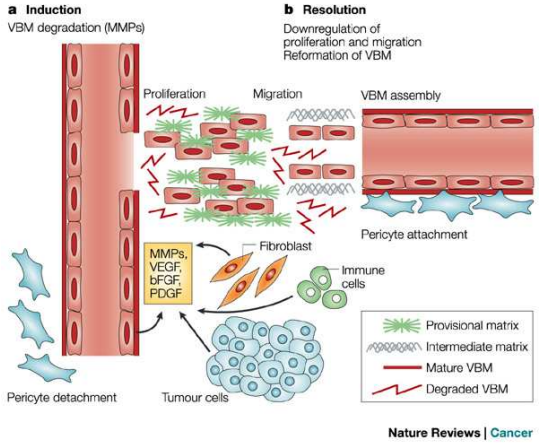
Proliferation phase: epithelisation

- on the surface of the wound, epithelial cells burst into mitotic activity within 24 to 72 hours
  - stimulated by growth factors (eg. EGF)
- epithelia / keratinocytes migrate and proliferate from the wound margins (and hair folicles)
- later, differentiation and stratification occurs





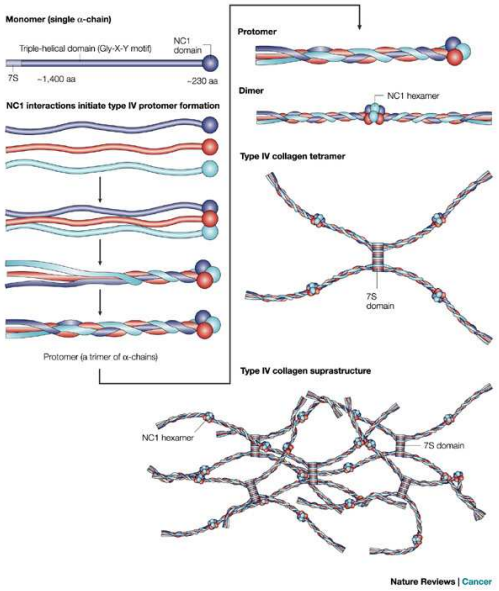
Proliferation phase: angiogenesis



- growth angiogenic factors (VEGF) released upon hypoxia stimuli
- budding and proliferation of endothelial cells

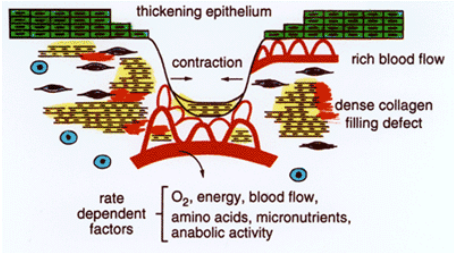
Proliferation phase: fibrotisation

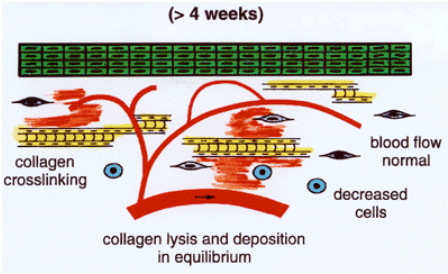
- fibroblasts proliferate in the deeper parts of the wound
  - begin to synthesize small amounts of collagen which acts as a scaffold for migration and further fibroblast proliferation
- granulation tissue, which consists of capillary loops supported in this developing collagen matrix, also appears in the deeper layers of the wound
- proteoglycans appear to enhance the formation of collagen fibers, but their exact role is not completely understood
- within two to three weeks, the wound can resist normal stresses, but wound strength continues to build for several months
- the proliferation phase lasts from 15 to 20 days and then wound healing enters the maturation phase



Maturation phase

- fibroblasts leave the wound and collagen is remodeled into a more organized matrix
- wound contracts
  - tensile strength of collagen increases for up to one year following the injury
  - while healed wounds never regain the full strength of uninjured skin, they can regain up to 70 to 80% of its original strength
- wound remodeling (scar maturation)
  - increasing collagen crosslinking, resulting in increased strength
  - action of collagenase to begin breaking down excess collagen accumulation
  - regression of the lush network of surface capillaries as metabolic demands diminish
  - decreasing proteoglycan and, in turn, wound water content





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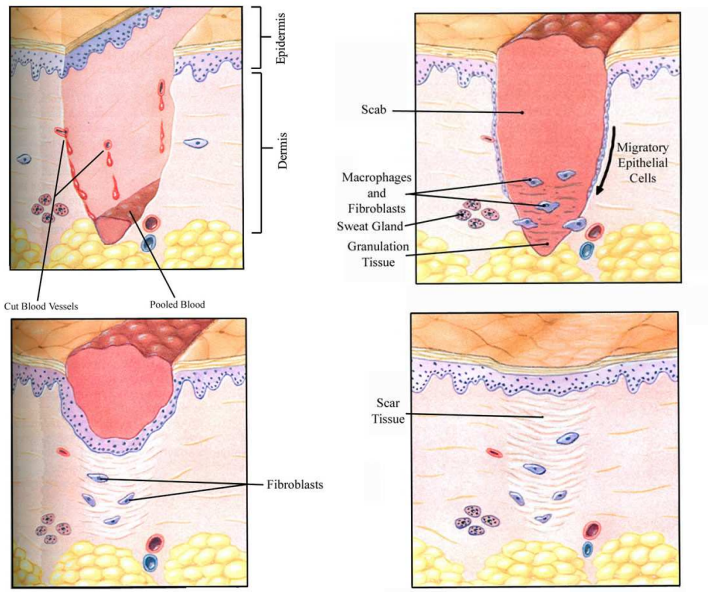
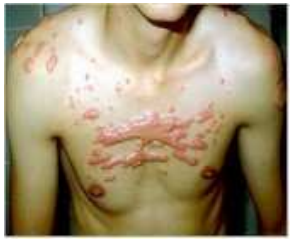


Abnormal acute wound healing

- keloid
  - abnormal scar that grows beyond the boundary of the original site of a skin injury
  - some ethnic groups are at more risk
    - 15-times more common in highly pigmented ethnic groups (African-American or Hispanic populations) rather than Caucasians
- hypertrophic scar
  - looks similar to a keloid
  - more common
  - don't get a big as keloids and may fade with time
  - occur in all racial groups



(3c) Chronic wound

- failure or delay of healing
- unresponsiveness to normal regulatory factors
- ethiopathogenic factors
  - local
    - repeated trauma
    - foreign body
    - poor perfusion/oxygenation (macro- and microvascular disease, neuropathy)
    - excessive/permanent infection
  - systemic
    - malnutrition
    - immunodeficiency / immunosupression
    - systemic disease (diabetes mellitus, Cushing d., …)
    - genetic causes

Wound healing vs. carcinogenesis