HEALING AND REPAIR

Kenneth Alonso, MD, FACP

Healing and repair

- Refers to the restoration of tissue architecture and function after an injury.
- Repair is a term used for parenchymal and connective tissues
- Healing is a term used for surface epithelia
- Regeneration by proliferation of uninjured cells AND maturation of tissue stem cells
- Driven by growth factors
- Dependent upon integrity of extracellular matrix
- Deposition of connective tissue to form a scar
- If it occurs in a space occupied by an inflammatory exudate, it is referred to as "organization."

Repair

•The ability of tissues to repair themselves is determined in part by their intrinsic proliferative capacity.

•<u>Continuously dividing (labile) tissues</u>

•Proliferation of mature cells and maturation of stem cells

- Hematopoietic cells in the marrow
- Surface epithelium (usually squamous)
- Cuboidal epithelium of ducts draining exocrine organs
- Columnar epithelium
- Uterus and fallopian tubes
- Transitional epithelium (urinary tract)

Repair

- Stable tissues
- G_0 (resting phase)
- Divide in response to injury or diminished cell mass
- Parenchyma of solid tissues
- Endothelium
- Smooth muscle
- Fibroblasts
- Limited capacity to regenerate (liver as an exception)

Repair

- Permanent tissues
- Terminally differentiated
- Majority of neurons
- Limited replication in some areas of adult brain
- Majority of cardiac muscle cells
- Normal stem cell replacement is 1%/year
- Skeletal muscle
- Satellite cells attached to endomysial sheath provide limited regenerative capacity

Role of stem cells

- Tissue stem cells are multipotent or lineage committed (not pluripotent or embryonal)
- OCT 3/4, SOX2, NANOG, and LIN28 genes must be activated in adult cells to induce pluripotency
- Examples of niches where found:
- Subventricular zone and dentate gyrus of brain (neural)
- Bulge region of hair follicle
- Limbus of cornea
- Crypt cells of the small intestine
- Canals of Herring in the liver
- Marrow and fat (mesenchymal)

Role of stem cells

- Characterized by self-renewal
- One daughter cell enters differentiation pathway; the second remains undifferentiated and maintains stem cell pool (Asymmetric division; stochastic differentiation)
- WNT pathway stimulates; respond to tension
- BMP pathway inhibits
- Activation is growth factor driven
- Growth factors activate signaling pathways

- WNT and YAP/TAZ have also been linked to the pathophysiology of fibrosis.
- Tissue mechanics have been shown to orchestrate Wnt-dependent human embryonic stem cell differentiation.

Growth factors

- Methods of delivery
- Endocrine
- Factors secreted into the blood and conveyed to a distant site
- Insulin-like factors I and II are examples of this.
- Paracrine
- The secretory product of one cell acts directly on another.
- Cell to cell distances are therefore important for paracrine factors to act efficiently.
- PDGF, TGF-α, and TGF-β

Growth factors

- Autocrine
- Perform self-regulatory functions.
- TGF-β is an example of an autocrine factor whose secretory cell is both the source and target of its activity.

Growth factor	Source	Wound healing related functions
PDGF	Platelets, macrophages, endothelial cells, injured cells	Chemotaxis, fibroblast proliferation, collagenase production
TGF-β	Macrophages, platelets, neutrophils, lymphocytes, fibroblasts, epithelial and endothelial cells, injured cells	Fibroblast proliferation, chemotaxis, collagen metabolism
EGF	Plasma, platelets, macrophages, epithelial cells	Epithelial cell proliferation, granulation tissue formation
TGF-α	Activated macrophages, platelets, epithelial cells, injured cells	Epithelial cell proliferation, granulation tissue formation
KGF	Fibroblasts	Epithelial cell proliferation
IL-1	Macrophages	Fibroblast proliferation
FGF	Pituitary, macrophages, fibroblasts, endothelial cells	Fibroblast proliferation, matrix deposition, wound contraction angiogenesis
TNF-α	Macrophages, T lymphocytes	Fibroblast proliferation
IGF-1	Plasma, liver, fibroblasts	Synthesis of sulfated proteoglycans and collagen, fibroblast proliferation
IFNs	Lymphocytes, fibroblasts	Inhibition of fibroblast proliferation and collagen synthesis

doi: 10.4103/0975-7406.100294

Growth factors

- EGF and TGF-α affect mesenchymal and epithelial cells
- Derived from transmembrane proteins and act through a paracrine mechanism on the EGF receptor.
- Wound macrophages contain significant amounts of TGF-α that add to the significance of this cell in the initial tissue response to injury.
- The main effect of TGF-α and EGF appears to be on granulation tissue development, with epidermal re-growth and modulation of angiogenesis being unique features of TGF-α activity

Growth factors

- PDGF is stored in the α granules of platelets and released after activation of the platelets at sites of tissue injury.
- Macrophages, endothelial cells, vascular smooth muscle cells, and fibroblasts also express PDGF
- Chemotactic for fibroblasts and monocytes as well as mitogenic for fibroblasts and vascular smooth muscle cells.
- The production of PDGF at wound sites is not constant, and increases in concentration have been correlated with augmented connective tissue formation.

Growth factors

- PDGF acts through paracrine and autocrine mechanisms that enable it to function not only as a stimulator of cellular activity but also in a homeostatic feedback fashion.
- Stimulate tyrosine kinase, and increase transcription of c-fos and c-myc
- Its effects on angiogenesis are indirect

Growth factors

- TGF-β
- Identified in a wide variety of cells including platelets, macrophages, bone cells, monocytes, lymphocytes, and platelets.
- Has both mitogenic effects as well as regulatory functions over matrix production.
- Chemotactic for fibroblasts and monocytes and is capable of stimulating or inhibiting fibroblasts.
- Stimulate angiogenesis through the induction of IL-1 and TNF-α production in macrophages

Growth factors

- Basic fibroblast growth factors (bFGFs)
- High affinity for heparin.
- Release of occurs due to the action of the enzyme heparinase found in platelets
- Found in many different tissues including endothelial cells, macrophages, and fibroblasts
- Chemotactic toward endothelial cells and leukocytes as well as mitogenic for endothelial cells.
- Initiates release of basement membrane degrading enzymes that liberate endothelial cells before new vessel formation.

Growth factors

- Derived from M1 monocytes and macrophages:
- Epidermal growth factor (EGF)
- Mitogenic for keratinocytes and fibroblasts
- Also produced by platelets
- Transforming growth factor (TGF-α)
- Stimulates cell proliferation (especially, hepatocytes)
- Also produced by keratinocytes and T cells.

Growth factors

- Derived from M1 monocytes and macrophages:
- Fibroblast growth factors (FGF)
- FGF1 (acidic) and FGF2 (basic)
- Chemotactic and mitogenic for fibroblasts
- Stimulates angiogenesis (FGF2)
- Stimulates extracellular matrix (ECM) protein synthesis
- Produced also by endothelial cells and mast cells

Growth factors

- Derived from M2 monocytes:
- Transforming growth factor (TGF-β)
- Chemotactic for fibroblasts
- Stimulates ECM protein synthesis
- Suppresses acute inflammation
- Inhibits epithelial cell proliferation
- Increase expression of INK4/ARF
- Increase expression of $p21^{cip1}$ and $p27^{kip1}$
- Also produced by keratinocytes

Growth factors

- Produced from mesenchymal cells:
- Vascular endothelial growth factor (VEGF)
- Isoforms A-D involved in angiogenesis
- Simulates proliferation of endothelial cells
- Increases vascular permeability
- Produced from fibroblasts:
- Keratinocyte growth factor (KGF or FGF7)
- Stimulate keratinocyte migration
- Stimulate keratinocyte proliferation
- Stimulate keratinocyte differentiation

Growth factors

- Derived from fibroblasts and stromal cells in the liver (mesenchymal origin)
- Hepatocyte growth factor (HGF, scattering factor)
- Enhances proliferation of hepatocytes (and other epithelial cells)
- Enhances proliferation of endothelial cells
- Increases cell motility

Growth factors

- Produced from Platelets:
- Platelet derived growth factor (PDGF)
- Chemotactic for:
- Neutrophils
- Macrophages
- Fibroblasts
- Smooth muscle cells
- Activates and stimulates proliferation of:
- Fibroblasts
- Endothelial cells
- Stimulates ECM protein synthesis

Growth factors

- PDGF also produced by:
- Macrophages
- Endothelial cells
- Smooth muscle cells
- Keratinocytes.
- Isoforms CC and DD require cleavage
- Isoforms BB and CC involved in hepatocyte regeneration and in wound contraction
- Stored in platelet granules

Cytokines

- IL-1 is chemotactic for neutrophils
- Produced by endothelial cells
- Stimulates metalloproteinases
- Stimulates production and release of acute phase reactants from liver
- The cytokine IL-4 sustains growth factor production

Cytokines

CYTOKINE	FUNCTION
IL-1	First cytokine to be released. Fever Neutrophil chemotaxis IgA secretion on mucosal surfaces
IL-2	Maintains T cell production and activity
IL-4	Shifts to Th2 cells Stimulate B cells Class switches to IgG and IgE Maintains growth factor production
IL-5	Class switch to IgA Eosinophil chemotaxis
IL-6	Stimulates production of acute phase reactants
IL-8	Neutrophil chemotaxis
IL-10	Inhibits Th1 cells
IL-12	Shifts to Th1 cells Activates NK cells
IFN-γ	Activates NK cells

Repair by connective tissue deposition

- If repair cannot be accomplished by regeneration alone, it occurs by replacement of injured cells with connective tissue.
- Patches rather than restores tissue
- Scar usually refers to wound healing
- Granulation tissue intermediate remodeling phase
- β-catenin upregulation facilitates epithelial-mesenchymal transformation

Steps in granulation tissue formation

- Angiogenesis
- VEGF driven
- Vasodilatation in response to NO
- Hypoxia inducible factor-1 (HIF-1) promotes new vessel formation, stimulates cell survival pathways, and enhances anerobic glycolysis
- Production stimulated by VEGF A
- VEGFR 2 critical receptor
- Increased vascular permeability associated with post-inflammation edema

Steps in granulation tissue formation

- Angiogenesis 1 and 2 growth factors lead to:
- Pericyte separation from vessel wall and
- Basement membrane break down, lead to sprout
- Endothelial cells migrate to sprout
- Remodel sprout into capillary tube

Steps in granulation tissue formation

- FGF2
- Stimulates endothelial cell proliferation
- Promotes fibroblast migration to site
- Promotes macrophage migration to site
- Activated macrophages clear dead tissue
- Produce growth factors and cytokines
- Stimulate fibrous tissue deposition
- PDGF recruits smooth muscle cells for vessels
- Notch signaling regulates branching and spacing of new vessels (through cross-talk with VEGF)

Steps in granulation tissue formation

- TGF-β stabilizes process
- Suppresses endothelial cell proliferation and migration
- Suppresses leukocyte migration
- Stimulates collagen and fibronectin synthesis
- Intact laminin necessary for repair
- With Type IV collagen, form basement membranes
- Enhances production of ECM proteins
- Integrins and scaffolding proteins
- Post-transcriptional activation of latent TGF-β regulates levels

Steps in granulation tissue formation

- Metalloproteinases degrade ECM to permit remodeling and extension of vascular tube
- Degrade Type III collagen for replacement by Type I
- Zinc dependent removal of Type III collagen
- Activity tightly controlled
- Produced as inactive precursors (zymogens)
- Activated by proteases at site (e.g., plasmin)
- Inhibited by specific tissue inhibitors (TIMPs)
- Produced by mesenchymal cells

Steps in granulation tissue formation

- Type III collagen is the initial collagen produced
- Tropocollagen is a triple helix of α-chains
- It is the structural unit of collagen
- Hydroxylation of proline and lysine requires ascorbic acid
- Hydroxyproline residues stabilize the triple helix
- Hydroxylysine residues oxidized to aldehyde and covalently cross-link the triple helix
- Copper is a co-factor with lysine oxidase
- Cross-linking increases tensile strength
- Type I collagen in skin, bone, tendons has greatest tensile strength

Steps in granulation tissue formation

- ADAMs (disintegrin and metalloproteinase)
- Plasma membrane anchored
- Cleave and release extracellular domains of cell-associated cytokines and growth factors

- Maturation and reorganization of connective tissue is end result of repair (scar)

Skin wound healing
Primary intention

- Injury only involves epithelial layers
- 24 hours clot
- 24-48 hours Neutrophils prominent
- 48 hours epithelial layers migrate from both edges and proliferate along the dermis
- Meet in midline beneath clot
- 48-72 hours Macrophages predominate
- Type III collagen evident at incision margins

Skin wound healing
Primary intention

- 120 hours Neovascularization
- Collagen bridges site
- Type I collagen
- Tensile strength 10% as compared to pre-injury
- With sutures in place, tensile strength is 70% that of pre-injury
- 30 days Scar comprises acellular tissue devoid of inflammation and adnexal cells
- Type III collagen
- Tensile strength 80% as compared to pre-injury

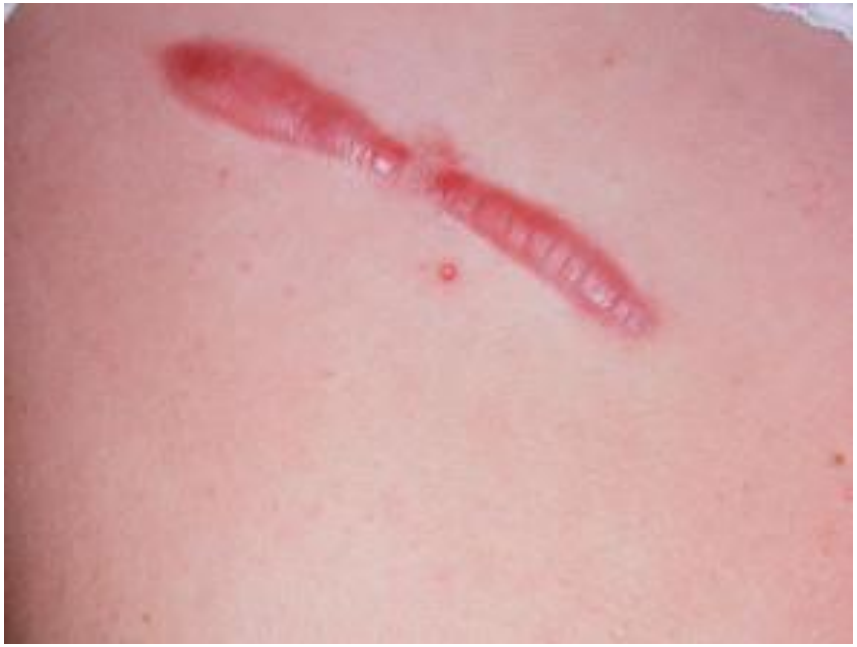
Skin wound healing
Secondary intention

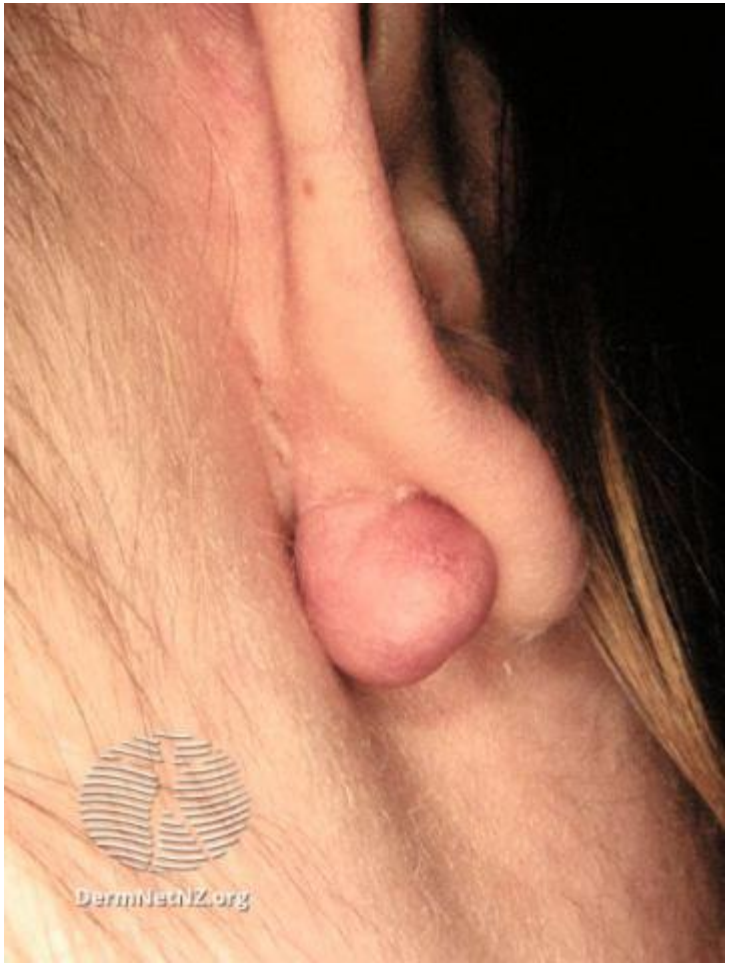
- Gaping wound
- Loss of dermal tissues
- More intense inflammatory response and granulation tissue formation
- Fibroblast conversion to myofibroblasts at wound periphery lead to contraction of wound site
- 90% wound closure by 6 weeks largely as result of contraction

Skin wound healing
Secondary intention

- Excessive ECM gives rise to a raised scar (hypertrophic)
- If the scar grows belong the boundaries of the original wound and does not regress, is a keloid
- Common in those of Mediterranean littoral as well as African ancestry
- Exuberant production of granulation tissues is “proud flesh”
- Occasionally may see disordered repair (fibromatosis)

Keloid





https://dermnetnz.org/topics/keloid-images/
Accessed 12/10/2019

Skin wound healing
Secondary intention

- Inadequate formation of granulation tissue or scar may lead to dehiscence (wound rupture)
- Usual cause is suture technique
- Subacute infection, COPD, anemia, obesity, poor nutrition, and diabetes mellitus are comorbidities
- If wounded area in region of neuropathic change, it is unlikely to heal (e.g., diabetics)

Wound closure

- Purse string closure
- The mechanism employs actomyosin ring contractions along the leading edge of the wound.
- The distribution of mechanical force generated by this contractile ring should decrease radially towards the centre.
- Likely to scar

Wound closure

- The use of subcutaneous/fascial tensile reduction sutures places the tension on the deep fascia and superficial fascia layer
- The wound edges may thus be joined naturally under very small tension, avoiding dermal sutures.
- The consequences of this suturing strategy are that wound edges are elevated, smooth, with minimal tension on the dermis.
- Less likely to scar.

Table 2 | Tissue strength during healing[24]	
Time after incision	**% of pre-incision breaking strength**
1 week	3
3 weeks	30
3 months	80

World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
Accessed 12/10/2019

Table 3 | Proportion of dehisced wounds that are infected	
Type of dehiscence	**Proportion of dehisced wounds that are infected**
Abdominal dehiscence[4,16,36]	52%–61%
Dehiscence following colorectal surgery[5]	36.7%
Sternal incision dehiscence[3]	49%
Episiotomy dehiscence[37]	Up to 80%

World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
Accessed 12/10/2019

- Mortality rates may be as high as 40% following dehiscence of an abdominal wound
- Facial sutures are generally removed at 6 days because of infection risk

Table 8 | Signs of progressing and impaired incision healing[8,14,91]			
Parameter	**Relationship to TIME framework***	**Signs that incisional healing is progressing well**	**Signs that healing is impaired**
Incision colour	**T**issue	■ Days 1–4: red ■ Days 5–14: bright pink ■ Day 15–1 year: pale pink, progressing to white or silver in light-skinned patients or to darker than usual skin colour in patients with darkly-pigmented skin	■ Days 1–4: may be red, tension in the incision line ■ Days 5–9: edges may be well-approximated and the tension remains ■ Days 10–14: if SWD does not occur, colour may remain red or progress to pink and may be followed ultimately by hypertrophic scarring
Healing ridge		■ Days 5–9: a healing ridge of thickened tissue indicating newly formed collagen can be felt about 1cm either side of the incision along its length, and persists into the remodelling phase	■ Lack of healing ridge
Peri-incisional area	**I**nfection/ inflammation	■ Signs of inflammation: - Mild oedema, erythema, warmth or skin discolouration that resolves by day 5 - Pain	■ Signs of inflammation may be absent in the first few days after surgery ■ Signs of inflammation and ongoing pain may be present for extended periods
Exudate	**M**oisture	■ Days 1–4: decreasing in volume from moderate to minimal and changing from sanguineous (blood) to serosanguineous (mixture of blood and serum) to serous (clear, amber serum) ■ Resolves by day 5	■ Exudate persists beyond days 1–4 ■ Exudate may be serosanguineous, serous or purulent (e.g. cloudy, green, yellow or brown)
Wound margins	**E**dge	■ Epithelial closure should be seen by day 4 along the entire incision ■ Approximated	■ Epithelial resurfacing may be only partially present or entirely absent ■ Area(s) of separation (SWD) may be present by day 14

World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
Accessed 12/10/2019

Table 9 | Assessment of SWD using the TIME framework (adapted from[93,96,97,99])		
Parameter	**Assess**	**Specifics**
Tissue	Location and extent of dehiscence	■ Location of the incision ■ Proportion of the incision affected ■ Number of areas of dehiscence ■ Presence of sutures/clips and condition (intact/broken)
	Depth of dehiscence	■ Partial or full-thickness dehiscence and tissue layers affected (see Figure 8, page 18); WUWHS SWD Grade (see Table 10, page 18) ■ Extension to or exposure of organs/bone/implant ■ Presence of undermining/tunnelling ■ For abdominal SWD, presence of evisceration
	Tissue viability	■ Condition of exposed tissues ■ Wound bed tissue types and proportions – e.g. of necrotic/devitalised tissue, slough and granulation tissue
	Dimensions	■ Dimensions of the dehisced area(s): maximum length, width, depth
Infection (or inflammation)	For local indicators of infection or inflammation	■ Clinical signs and symptoms ■ See Box 5 and Box 6, page 13, and Box 8, page 17, for signs and symptoms of acute and chronic infection ■ N.B. In patients who are immunosuppressed, signs and symptoms may be less obvious
Moisture	Exudate/drainage colour, consistency, type and odour	■ Purulent (cream, yellow or green) or haemopurulent (red, brown) may indicate infection ■ Yellow or brown exudate may indicate a urinary or enteric fistula ■ Malodour may indicate infection or fistula
	Exudate/drainage level	■ Indications of the level of exudate production can be gained from the condition of the current dressing (i.e. a dry dressing indicates low exudate levels; a saturated or leaking dressing indicates higher levels) and the appearance of the wound bed
Edge	Edges of dehisced area	■ In long-standing areas of dehiscence, the edges may become undermined
	Colour and condition of the surrounding skin	■ Signs of dermatological conditions that may affect healing – e.g. radiation dermatitis ■ Signs of spreading infection – e.g. spreading erythema, warmth and oedema ■ Periwound maceration may indicate high exudate/drainage levels and/or inadequate absorbency of the dressing

World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
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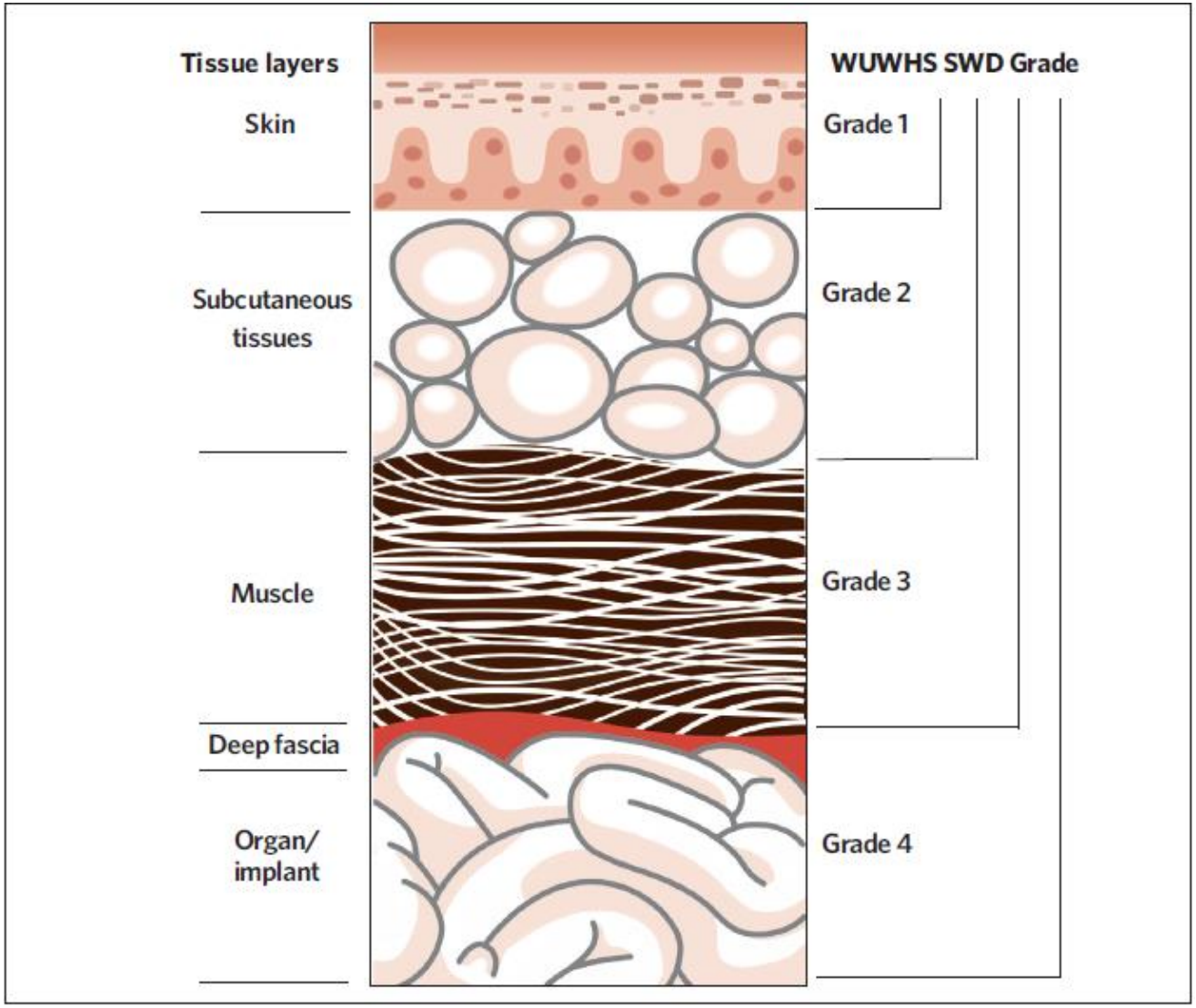

World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
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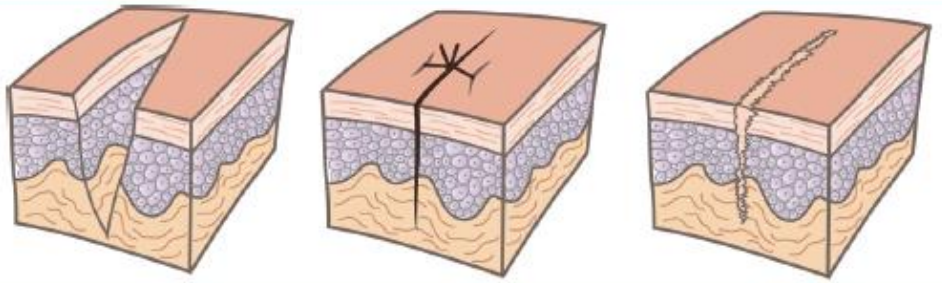
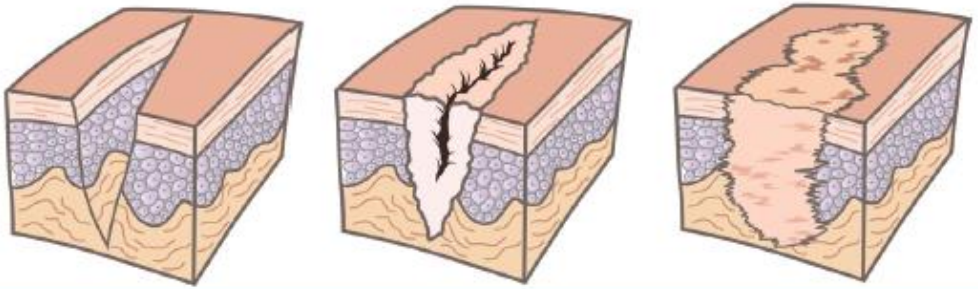
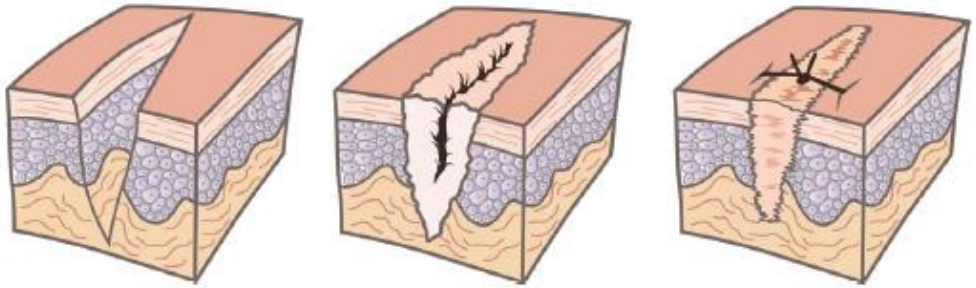
Primary closure
The edges of the incision are closely opposed, e.g. by suturing, stapling or taping, to allow healing by primary intention



Secondary closure
The incision is left open and heals by secondary intention as new tissue infills from the base and sides of the wound



Delayed primary closure
The incision is left open for up to several days or sometimes weeks, to allow for treatment of infection/contamination, removal (sequentially if necessary) of non-viable tissue, and/or for resolution of swelling, before proceeding to primary closure or closure with a flap/graft



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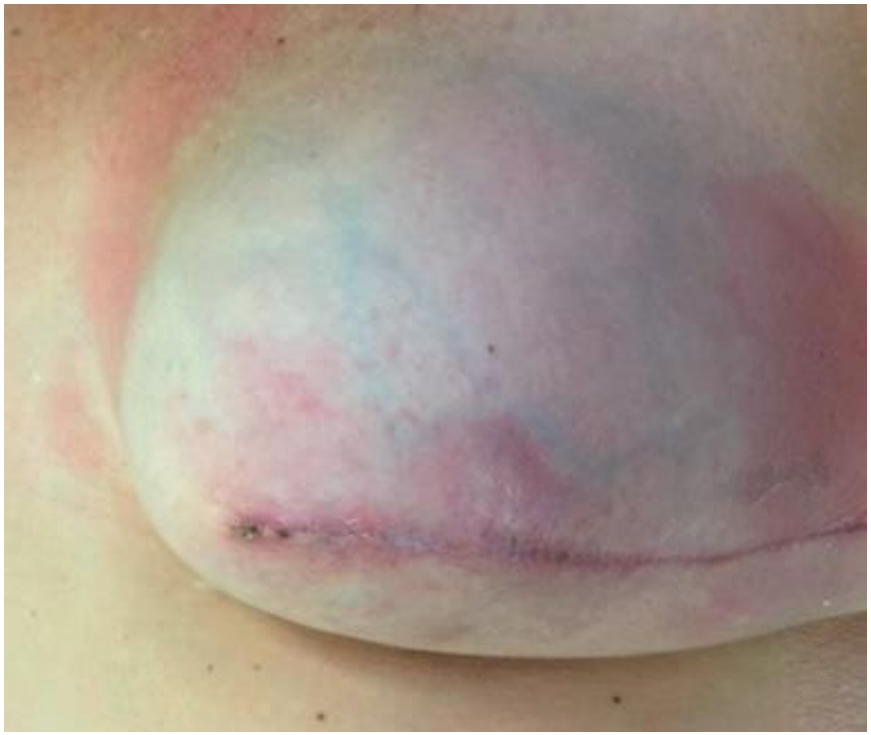
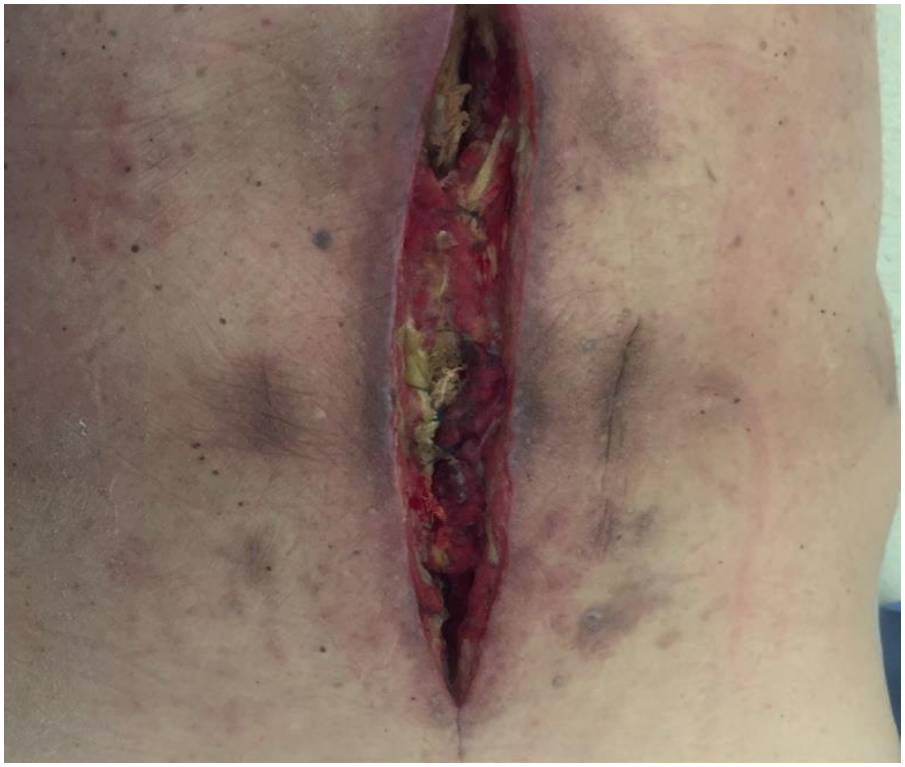
Grade 2 dehiscence



Grade 3 dehiscence



World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
Accessed 12/10/2019

Superficial fibromatoses

- Characterized by nodular or poorly defined broad fascicles of mature-appearing myofibroblasts surrounded by abundant dense collagen.
- Trisomy 3 and trisomy 18 common abnormalities.
- The palmar variant (Dupuytren contracture), there is irregular or nodular thickening of the palmar fascia (bilaterally in 50%).
- Attachment to the overlying skin causes puckering and dimpling.
- At the same time, a slowly progressive flexion contracture develops, mainly of the fourth and fifth fingers of the hand.

Fibromatoses

- With the plantar variant flexion contractures are uncommon and bilateral involvement is infrequent.
- In penile fibromatosis (Peyronie disease), a palpable induration or mass appears usually on the dorsolateral aspect of the penis.
- It may cause eventually abnormal curvature of the shaft or constriction of the urethra, or both.
- 10% of all fibromatoses.

Deep seated fibromatoses

- Deep-seated fibromatoses (desmoid tumors) lie in the borderland between nonaggressive fibrous tumors and low-grade fibrosarcomas.
- 2^{nd}-4^{th} decades
- No sex predilection
- Commonly present as large, infiltrative masses that frequently recur after incomplete excision.

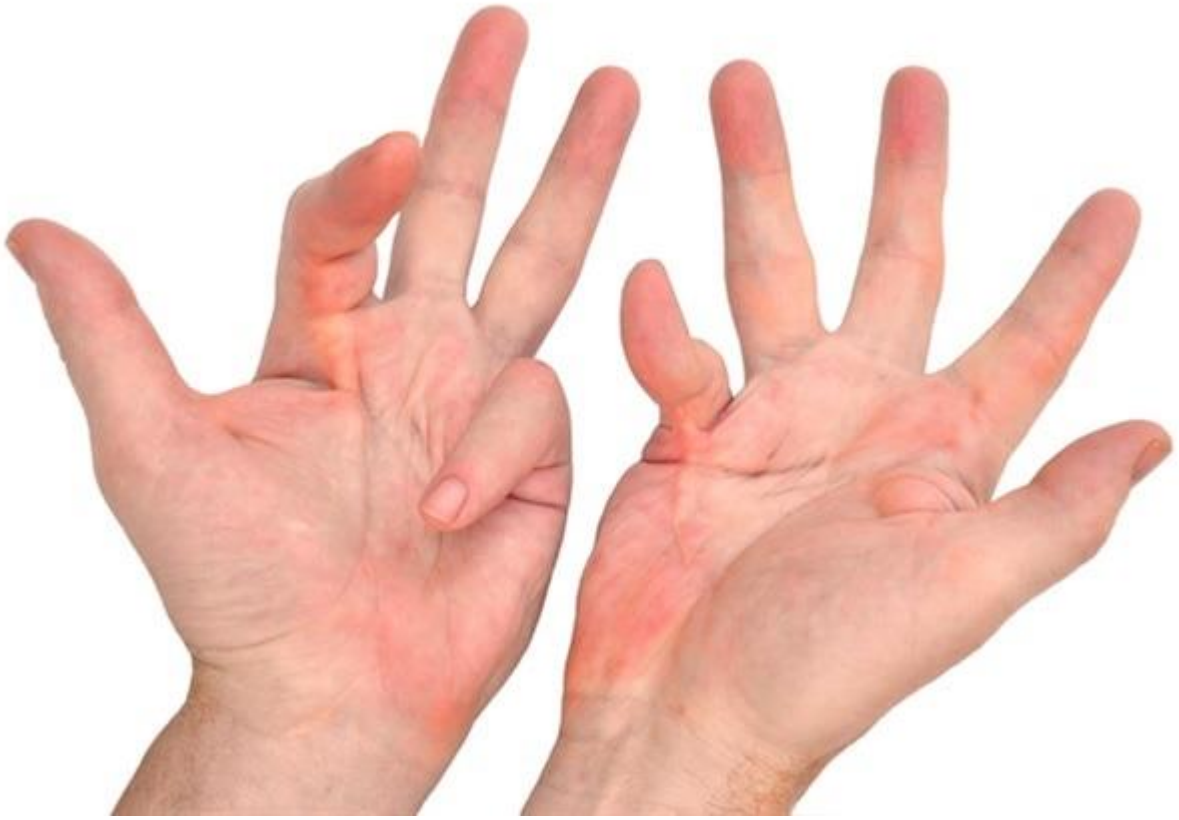
Deep seated fibromatoses

- Commonly present as large, infiltrative masses that frequently recur after Composed of plump fibroblasts arranged in broad sweeping fascicles that infiltrate to the adjacent tissue.
- Mitoses are usually infrequent.
- Trapped regenerating muscle cells may be mistaken for multi-nucleated giant cells

Desmoid tumor

- Extra-abdominal arise principally in the musculature of the shoulder, chest wall, back, and thigh.
- Abdominal desmoids generally arise in the musculo-aponeurotic structures of the anterior abdominal wall in women during or after pregnancy.
- Intra-abdominal desmoids tend to occur in the mesentery or pelvic walls, often in patients having familial adenomatous polyposis (Gardner syndrome).
- Mutations in the APC gene at 5q22.2 or β-catenin gene at 3p22.1 (both in the WNT pathway).
- <u>Do not metastasize.</u>

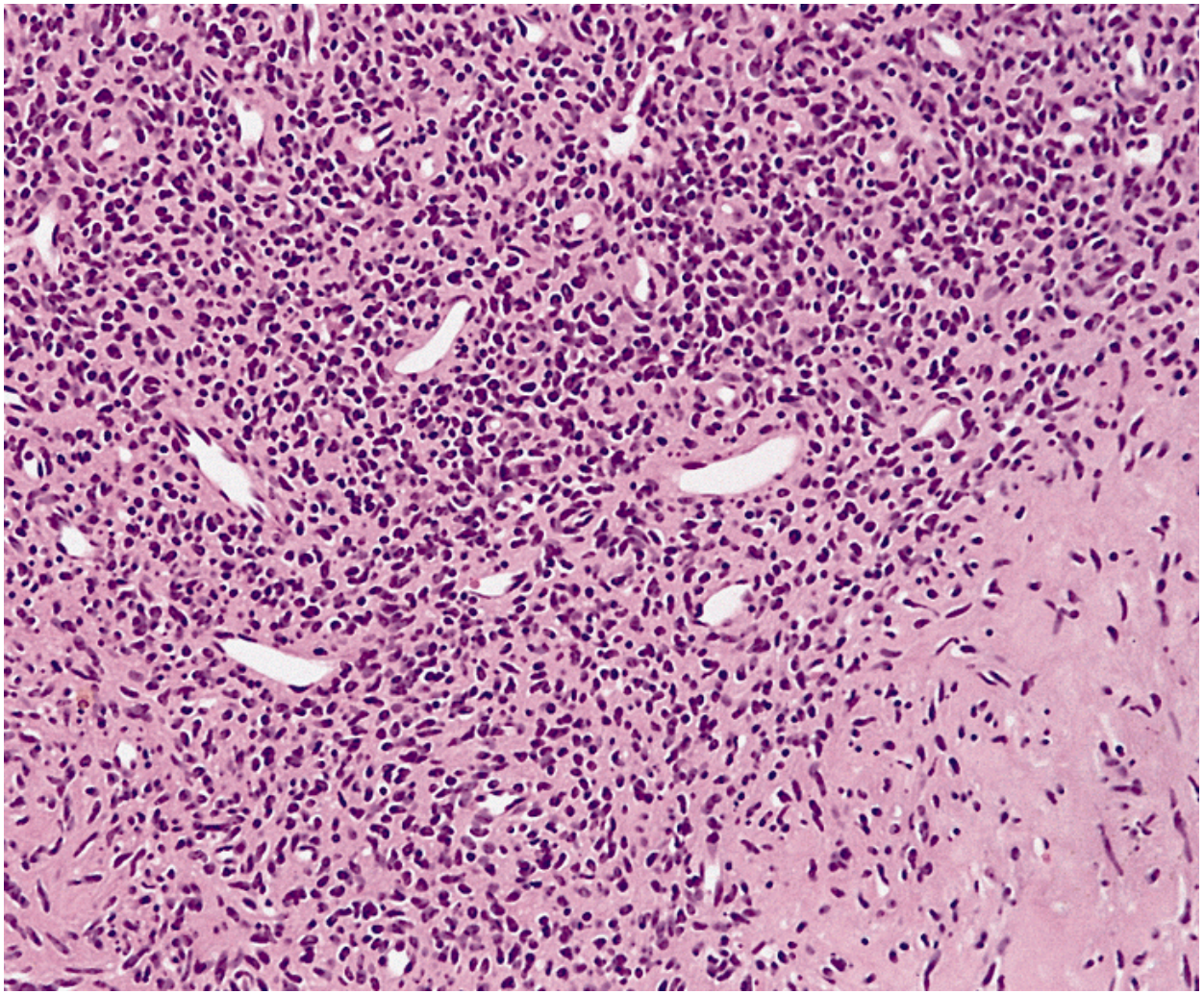
Palmar fibromatosis



Excessive scar formation with contraction may lead to deformity (e.g., palm)

https://www.news-medical.net/health/Dupuytrens-Contracture-(Palmar-Fibromatosis).aspx
Accessed 12/10/2019

Palmar fibromatosis



Excessive proliferation of fibroblasts adjacent to area of fibrosis

Fig. 2-45R Kempson, Richard L., Fletcher, Christophr DM, Evans, Harry L, Hendrickson, Michael R, and Sibley, Richard K, Tumors of the Soft Tissues, Fascicle 30, Atlas of Tumor Pathology. 3rd Series. Armed Forces Institute of Pathology, Washington, DC (2001).

Desmoid

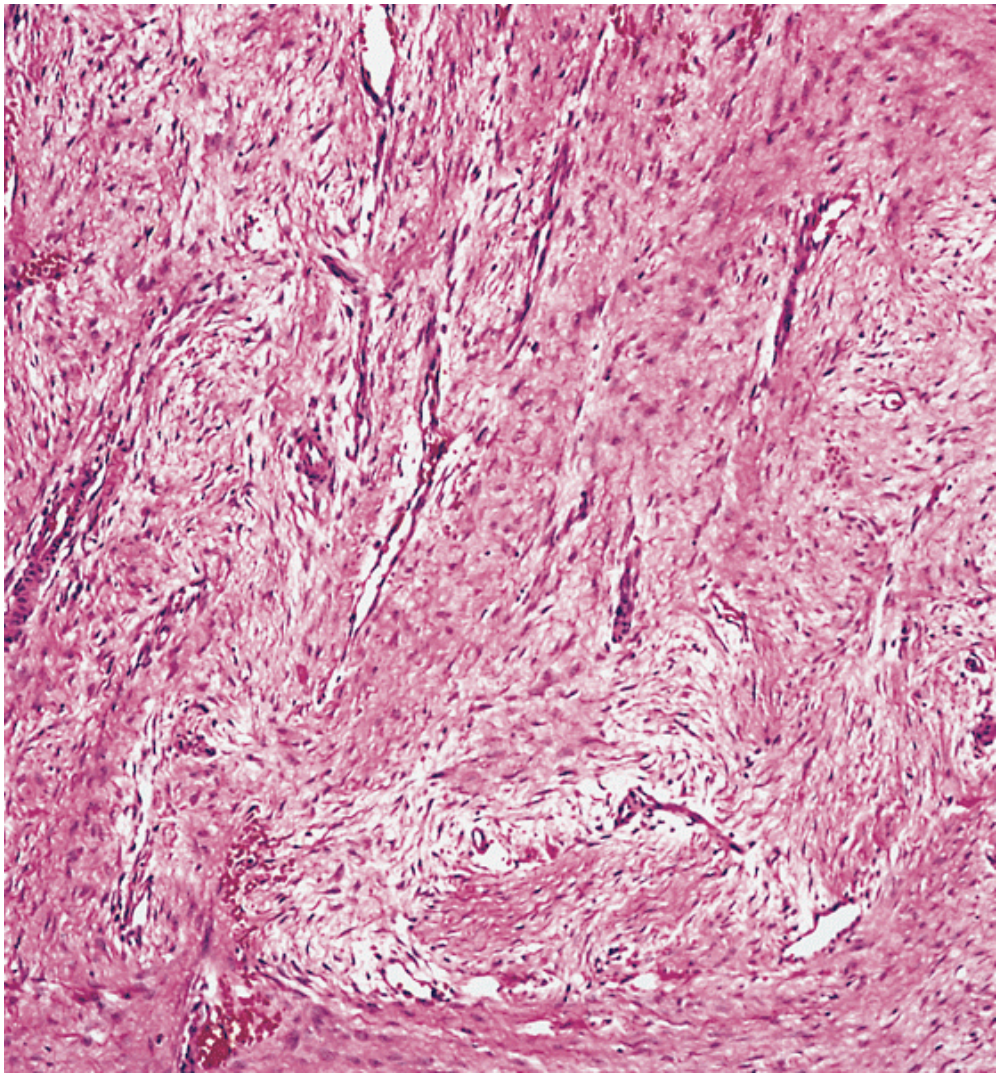
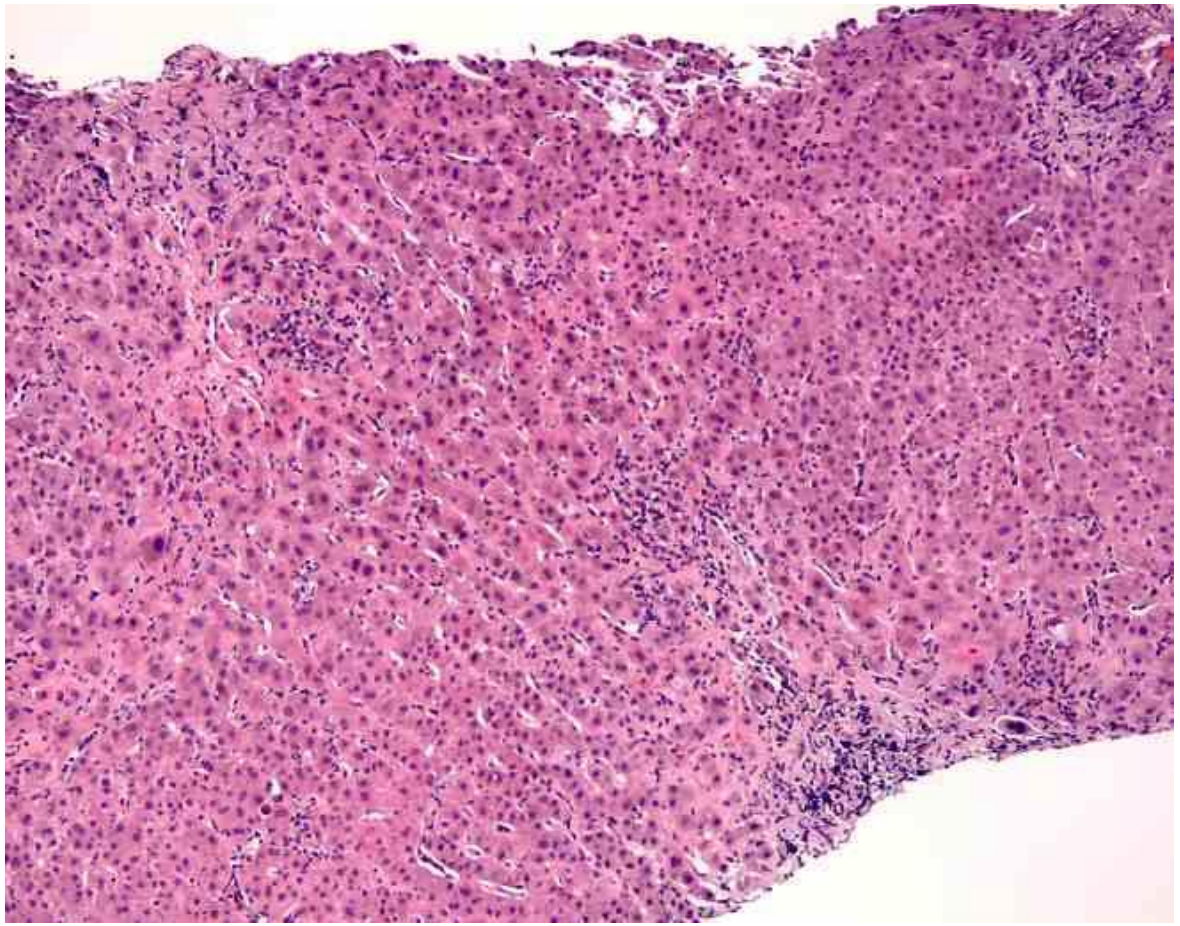


Fig. 2-49L Kempson, Richard L., Fletcher, Christophr DM, Evans, Harry L, Hendrickson, Michael R, and Sibley, Richard K, Tumors of the Soft Tissues, Fascicle 30, Atlas of Tumor Pathology. 3rd Series. Armed Forces Institute of Pathology, Washington, DC (2001).

Parenchymal repair

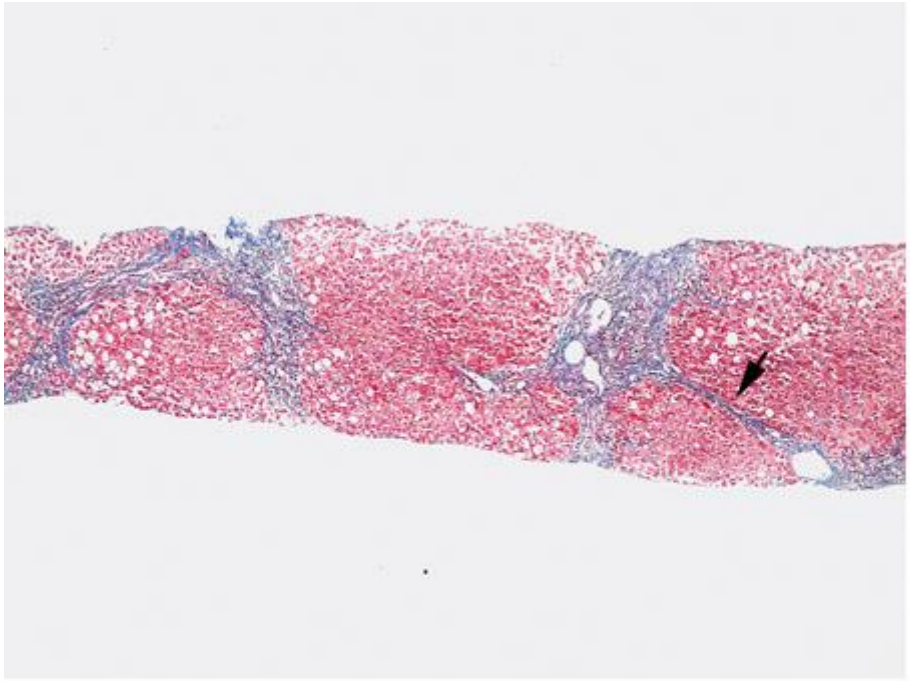
- Liver
- Regeneration of hepatocytes
- IL 6 produced and released by Kuppfer cells make hepatocytes competent to receive growth factor signals
- In severe injury, nodular regeneration
- Lack sinusoids and portal triads
- Fibrosis around portal triads with bridging between portal triads or to or between central veins
- Potential for cirrhosis

Chronic hepatitis



https://tpis.upmc.com/tpislibrary/images/H00190dmod.jpg
Accessed 12/04/2019

Chronic persistent hepatitis with bridging fibrosis



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

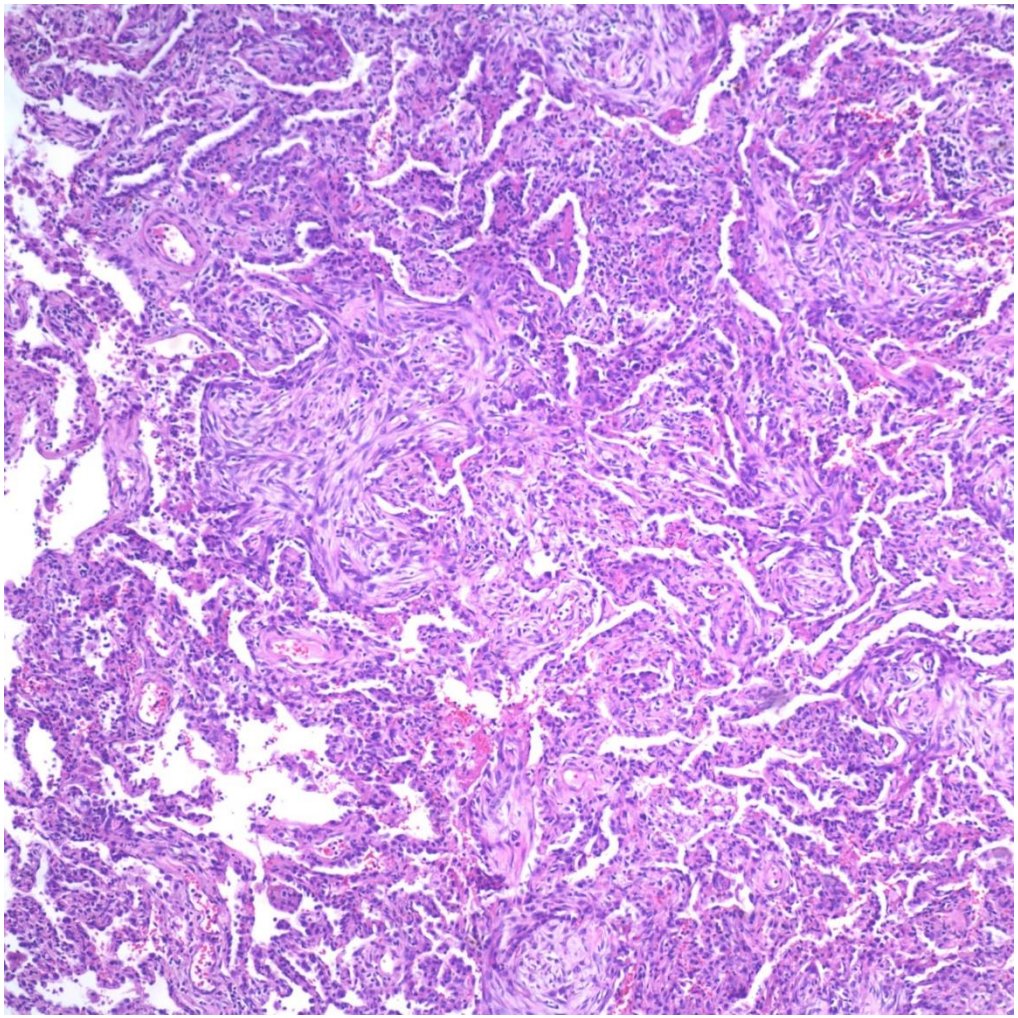
Fibrosis extends from portal area to portal area without loss of the limiting plate and with minimal inflammation. Masson Trichrome 10x

Fig. e26-12 Accessed 03/01/2010

Parenchymal repair

- Lung
- Type II pneumocytes replace damaged Type I and Type II pneumocytes
- Produce surfactant
- Fibrosis along alveolar septae

Organizing pneumonia



https://media.clinicaladvisor.com/images/dsm/ch4359.fig5.jpg
Accessed 12/04/2019

Parenchymal repair

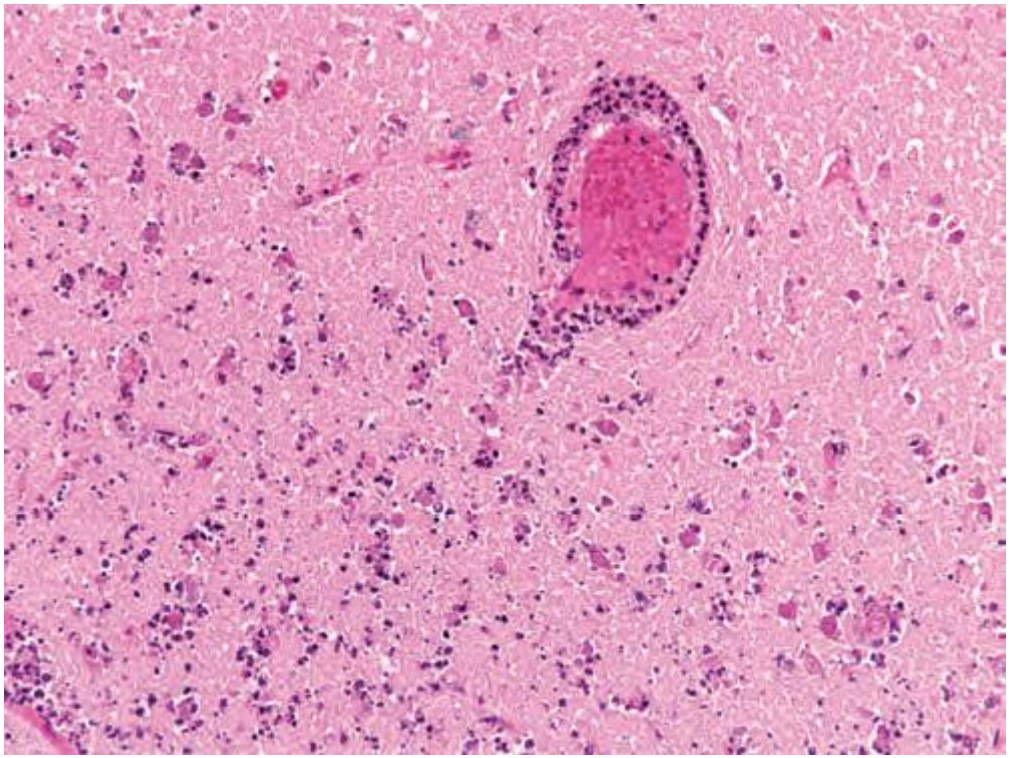
- Brain
- Microvacuolization of neurons followed by eosinophilia of neuronal cytoplasm (“Red neurons”)
- Neuronal bodies shrink; pyknosis and loss of nucleolus follow
- Occur later in astrocytes and oligodendrocytes.
- Myelinated fibers (axons) begin to disintegrate.
- Tau proteins from ruptured microtubules may aggregate, migrate, and further injure
-

Parenchymal repair

- Microglial cells (macrophages) prominent by 48 hours and become the predominant cell type over time.
- Mesoderm derived cells
- React by elongating nuclei (rod cells as in neurosyphillis)
- Aggregate around foci of necrosis
- Phagocytize neurons
- May persist in the lesion for months or years

Parenchymal repair

Neutrophil infiltration of injury site begins at edges of site where vascular supply has remained intact.



Frosch, MP, Anthony, DC, De Girolami, U, “The Central Nervous System,” in Kumar, V, Abbas, AK, Aster, JC, (eds), Robbins and Cotran Pathologic Basis of Disease (9th ed.), Elsevier. Philadelphia. (2015) Fig. 28-13C Accessed 10/25/2019

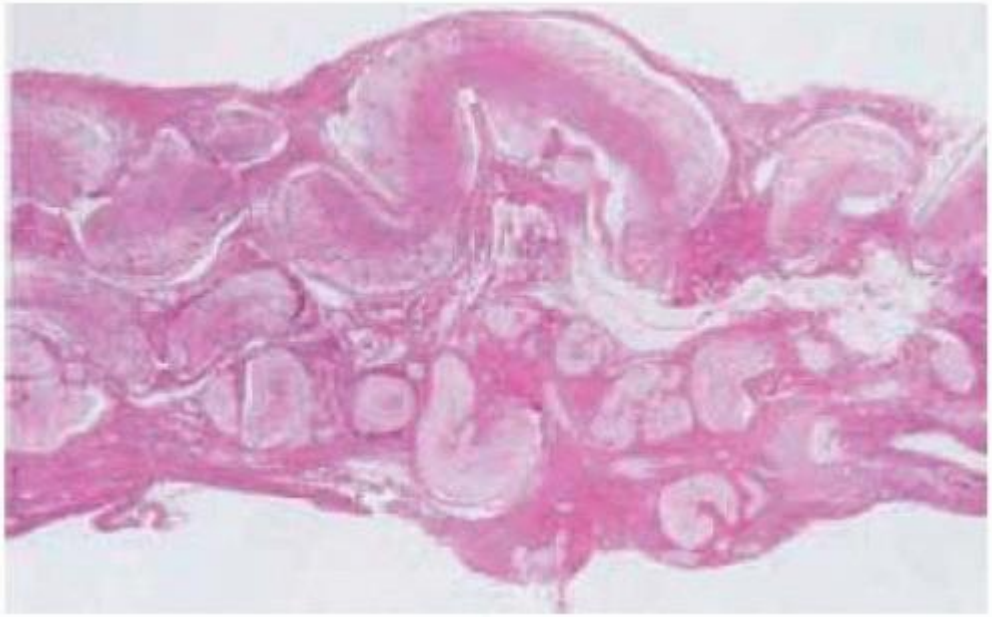
Parenchymal repair

- Peripheral nerve
- Distal degeneration of axon and myelin sheath (Wallerian degeneration)
- Proximal degeneration to node of Ranvier
- Schwann cells and macrophages phagocytize debris
- Muscle atrophy at 15 days
- Nerve cell body swells; Nissl bodies disappear centrally; nucleus to periphery

Parenchymal repair

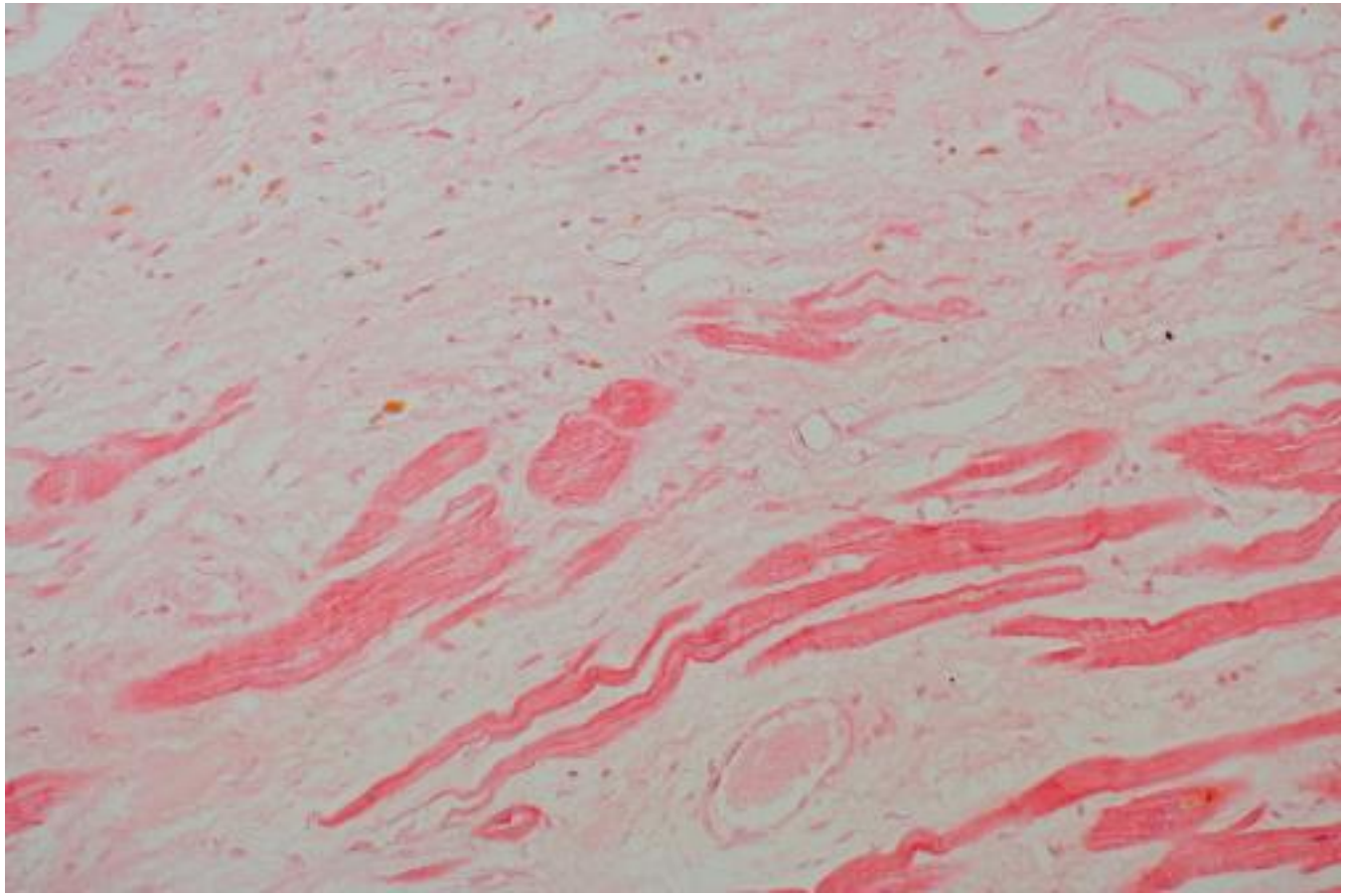
- Then, Schwann cells proliferate in distal stump
- Axonal sprouts from proximal stump extend distally to distal stump (under Schwann cell guidance)
- Axon growth 2-3 mm/day
- Demyelination
- Nerve eventually re-innervated
- Disordered organization gives rise to plexiform neurofibroma
- The heart heals only by scarring

Plexiform neurofibroma



https://entokey.com/wp-content/uploads/2016/08/DA1-C7-FF11.gif
Accessed 12/04/2019

Myocardium

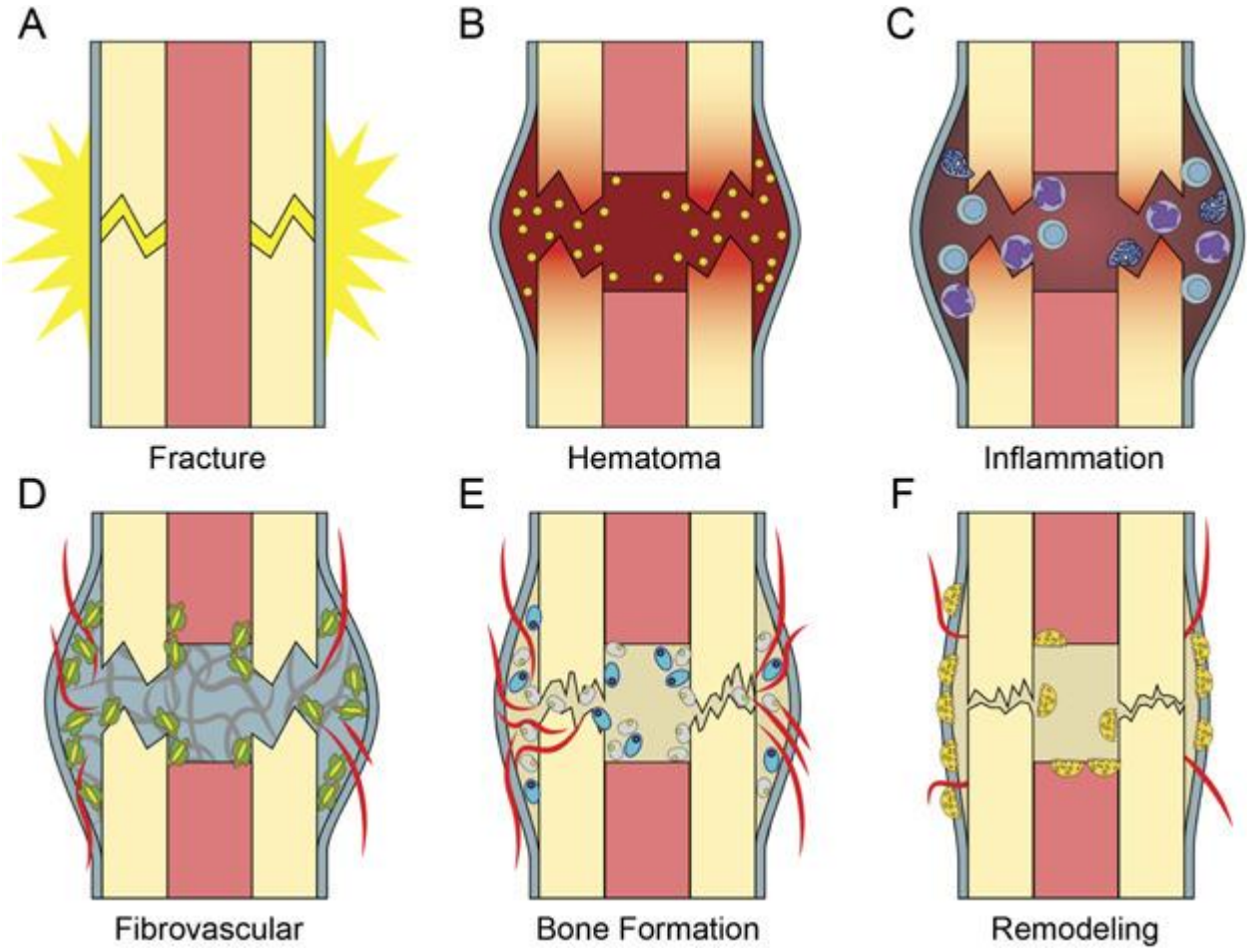


Two months post infarct. Little muscle remaining. Fibrous tissue deposition marked.

https://webpath.med.utah.edu/CVHTML/CV326.html
Accessed 12/10/2019

BONE HEALING

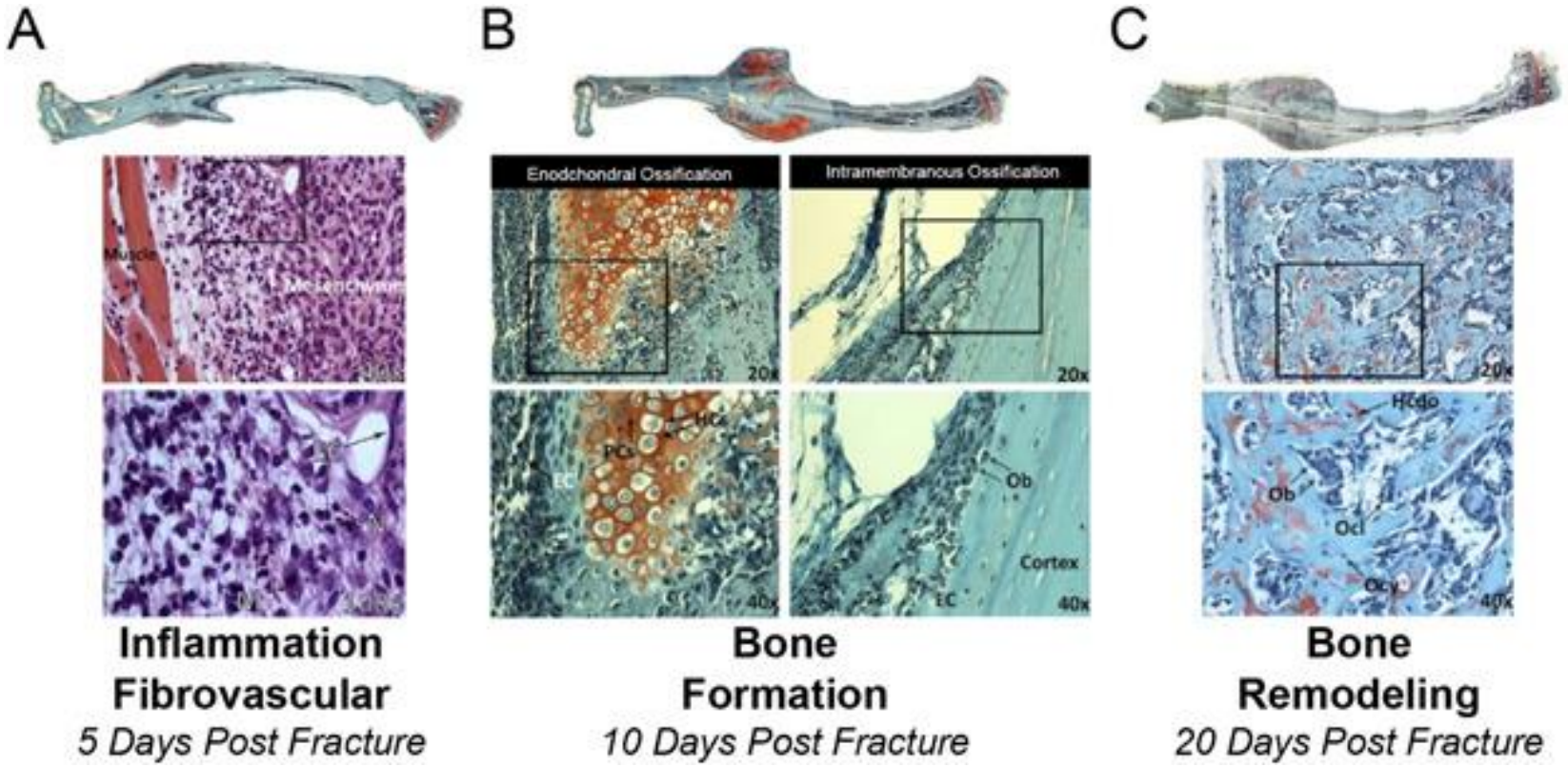
Cellular biology of fracture healing



Fracture healing is temporally-defined process.

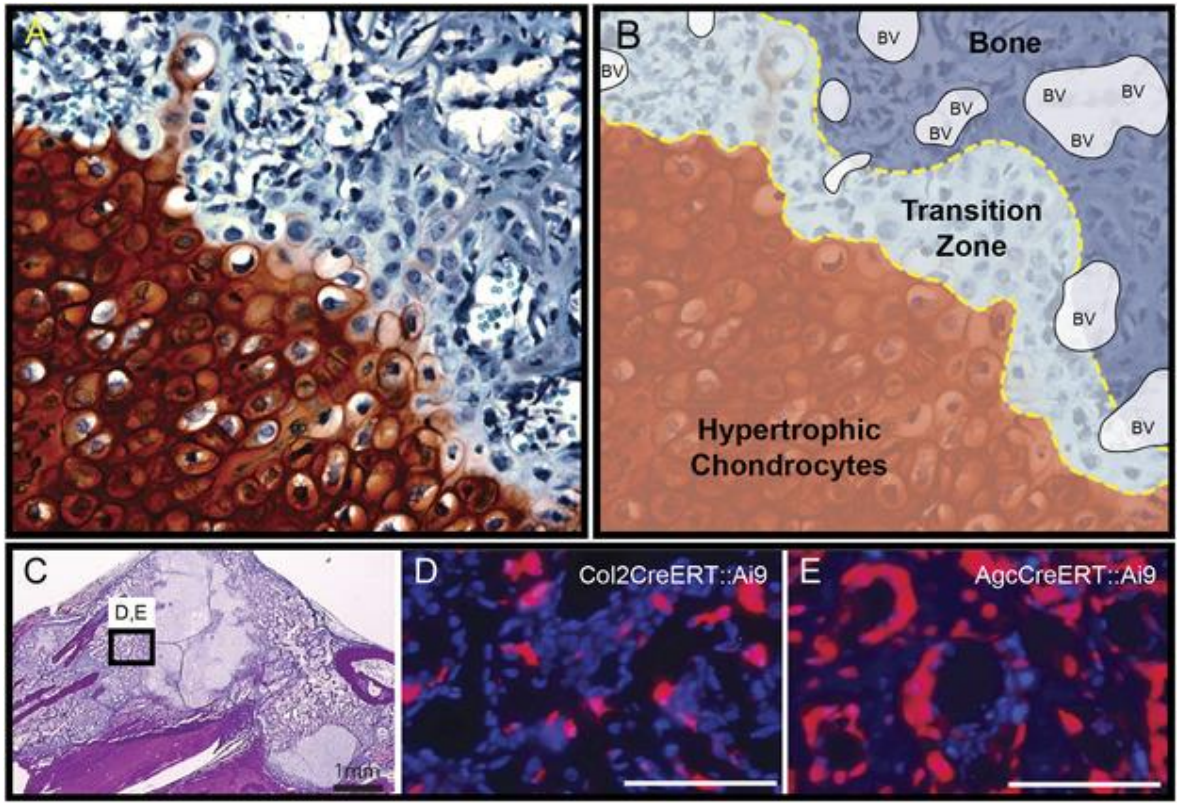
Journal of Orthopaedic Research, Volume: 37, Issue: 1, Pages: 35-50, First published: 28 October 2018, DOI: (10.1002/jor.24170)

Cellular biology of fracture healing



(A) undifferentiated mesenchymal cells are present in the callus and areas of inflammation remain (B) 10 days post-fracture there is both endochondral ossification (red staining, safranin-o stains cartilage) and intramembranous bone formation occurring. (C). An extensive network of primary bone has formed and endochondral ossification is complete.

Journal of Orthopaedic Research, Volume: 37, Issue: 1, Pages: 35-50, First published: 28 October 2018, DOI: (10.1002/jor.24170)



Hypertrophic chondrocytes develop into osteoblasts and osteocytes

Journal of Orthopaedic Research, Volume: 37, Issue: 1, Pages: 35-50, First published: 28 October 2018, DOI: (10.1002/jor.24170)

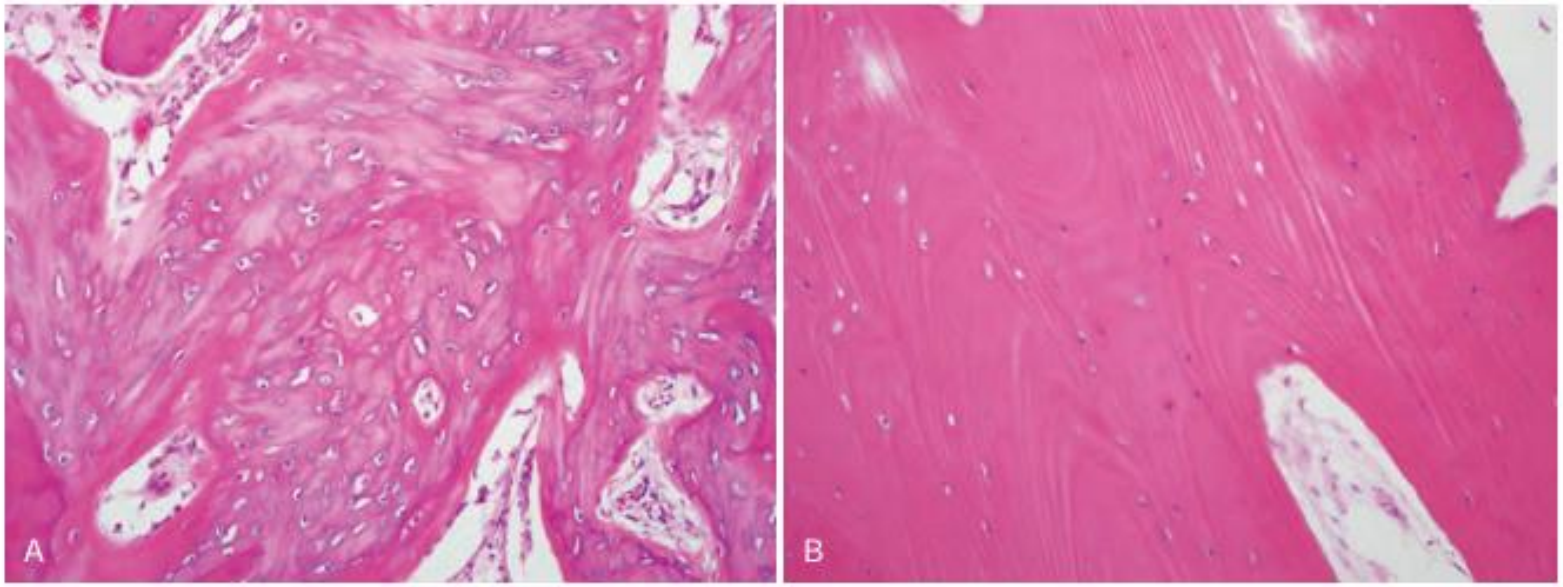
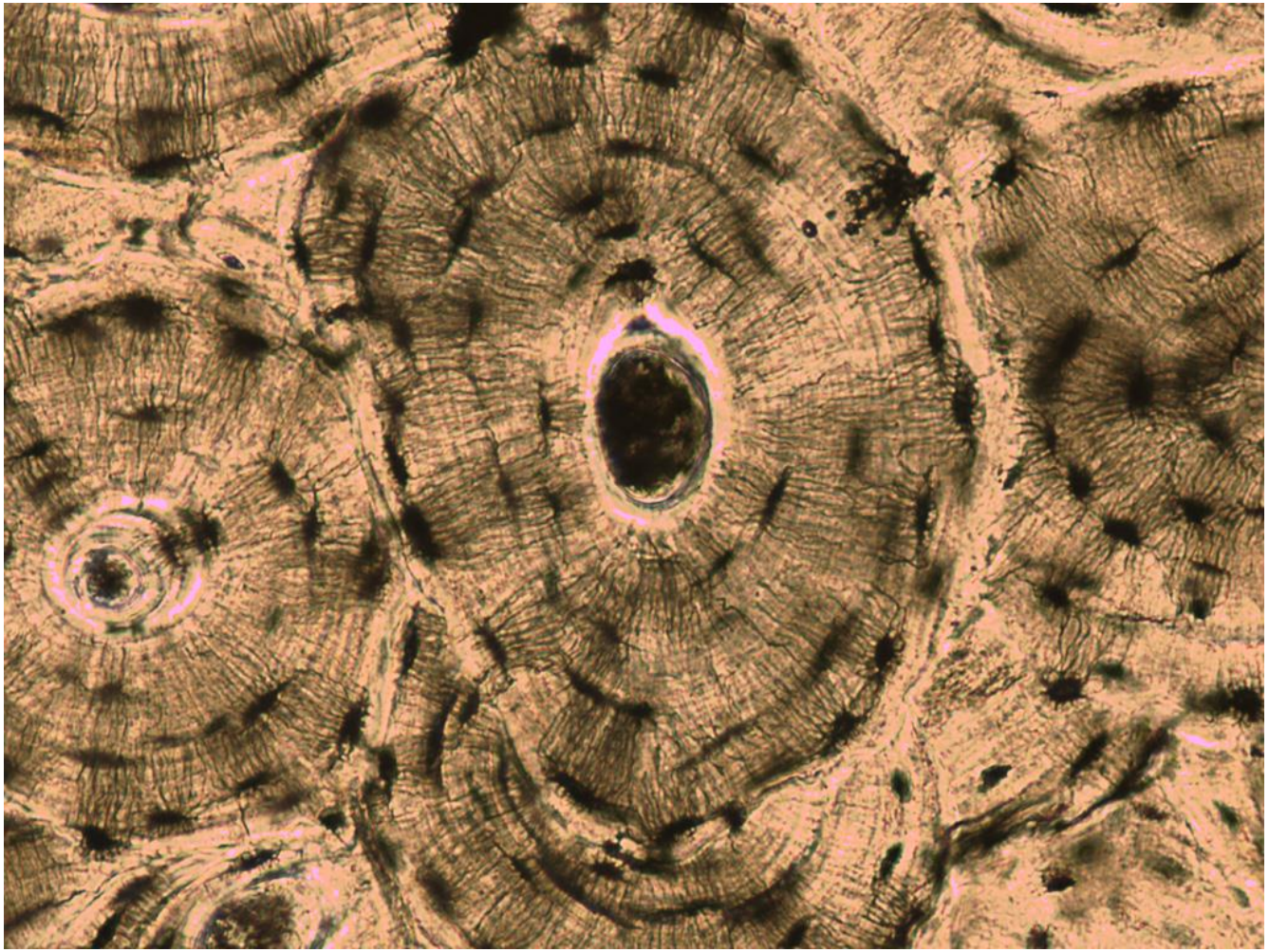


Figure 26-1 Woven bone **(A)** is more cellular and disorganized than lamellar bone **(B)**.



Osteon with Haversian canal in center

http://medcell.med.yale.edu/histology/bone_lab/images/haversian_system.jpg

Accessed 05/10/2020

Healing of bone fracture

- Following the initial trauma, bone heals by either direct intramembranous or indirect fracture healing, which consists of both intramembranous and endochondral bone formation.
- The most common pathway is indirect healing as direct bone healing requires an anatomical reduction and rigidly stable conditions.
- When such conditions are achieved, the direct healing cascade allows the bone structure to immediately regenerate anatomical lamellar bone and the Haversian systems without any remodeling steps necessary.

Contact healing

- Bone on one side of the cortex must unite with bone on the other side of the cortex to re-establish mechanical continuity.
- Under these conditions, osteoclasts which cross the fracture line, generate longitudinal cavities.
- These cavities are later filled by bone produced by osteoblasts residing at the rear of the juncture.
- This results in the simultaneous generation of a bony union and the restoration of Haversian systems formed in an axial direction.

Contact healing

- The re-established Haversian systems allow for penetration of blood vessels carrying osteoblastic precursors.
- The bridging osteons later mature by direct remodeling into lamellar bone resulting in fracture healing without the formation of periosteal callus.

Gap healing

- Bony union and Haversian remodeling do not occur simultaneously.
- It occurs if stable conditions and an anatomical reduction are achieved, although the gap must be less than 1 mm.
- In this process the fracture site is primarily filled by lamellar bone oriented perpendicular to the long axis, requiring a secondary osteonal reconstruction.

Gap healing

- The primary bone structure is then gradually replaced by longitudinal revascularized osteons carrying osteoprogenitor cells which differentiate into osteoblasts and produce lamellar bone on each surface of the gap.
- This lamellar bone, however, is laid down perpendicular to the long axis and is mechanically weak.

Gap healing

- This initial process takes approximately 3 and 8 weeks, after which a secondary remodeling resembling the contact healing cascade takes place.
- Although not as extensive as endochondral remodeling, this phase is necessary in order to fully restore the anatomical and biomechanical properties of the bone.

Healing of bone fracture

- Indirect healing
- Involves an acute inflammatory response and the recruitment of mesenchymal stem cells in order to generate a primary cartilaginous callus.
- This primary callus later undergoes revascularization and calcification, and is finally remodeled to fully restore a normal bone structure.

Healing of bone fracture

- Immediately following the trauma, a hematoma is generated and consists of cells from both peripheral and intramedullary blood, as well as bone marrow cells.
- The injury initiates an inflammatory response which is necessary for the healing to progress.
- The response causes the hematoma to coagulate in between and around the fracture ends, and within the medulla forming a template for callus formation.

Acute response

- TNF-α concentration has been shown to peak at 24h and to return to baseline within 72h post trauma.
- During this time-frame TNF-α is expressed by macrophages and other inflammatory cells, and it is believed to mediate an effect by inducing secondary inflammatory signals, and act as a chemotactic agent to recruit necessary cells.
- TNF-α also induces osteogenic differentiation of mesenchymal stem cells (MSC).

Acute response

- These effects are mediated by activation of the two receptors TNFR1 and TNFR2 which are expressed on both osteoblasts and osteoclasts.
- However, TNFR1 is always expressed in bone whereas TNFR2 is only expressed following injury

Acute response

- IL-1 and IL-6 are believed to be most important cytokines for fracture healing.
- IL-1 expression overlaps with that of TNF-α with a biphasic mode
- It is produced by macrophages in the acute phase of inflammation and induces production of IL-6 in osteoblasts
- Promotes the production of the primary cartilaginous callus, and also promotes angiogenesis at the injured site by activating either of its two receptors

Acute response

- IL-6 is only produced during the acute phase and stimulates angiogenesis, vascular endothelial growth factor (VEGF) production, and the differentiation of osteoblasts and osteoclasts.

- BMP-2 is essential for bone repair
- BMP-7 may plays a more important role in the recruitment of progenitor cells.

Acute response

- Stromal cell-derived factor-1(SDF-1) and its G-protein-coupled receptor CXCR-4 form an axis (SDF-1/CXCR-4) that is a key regulator of recruiting and homing specific MSCs to the site of trauma.
- SDF-1 expression is increased at the fracture site, especially in the periosteum at the edges of the fracture.
- SDF-1 has a specific role in recruiting CXCR-4 expressing MSCs to the injured site during endochondral fracture healing

Acute response

- Hypoxia inducible factor-1α (HIF-1α) induces the production of VEGF in the revascularization process
- Hypoxic gradients regulate MSC progenitor cell trafficking by HIF-1

Healing phase

- Following the formation of the primary hematoma, a fibrin-rich granulation tissue forms.
- Within this tissue, endochondral formation occurs in between the fracture ends, and external to periosteal sites.
- These regions are mechanically less stable
- The cartilaginous tissue forms a soft callus which gives the fracture a stable structure.

Healing phase

- Peak of soft callus formation occurs 7–9 days post trauma with a peak in both type II procollagen and proteoglycan core protein extracellular markers.
- At the same time, an intramembranous ossification response occurs subperiostally directly adjacent to the distal and proximal ends of the fracture, generating a hard callus.
- It is the final bridging of this central hard callus that ultimately provides the fracture with a semi-rigid structure which allows weight bearing

Healing phase

- The Wnt-family is thought to regulate the differentiation of pluripotent MSCs into the osteoblastic lineage, and, at later stages of development, to positively regulate osteoblastic bone formation.
- As fracture callus chondrocytes proliferate, they become hypertrophic and the extracellular matrix becomes calcified.
- Both osteoblasts and hypertrophic chondrocytes express high levels of VEGF
- Promote the invasion of blood vessels and transforming the avascular cartilaginous matrix into a vascularized osseous tissue.

Healing phase

- A cascade orchestrated primarily by macrophage colony-stimulating factor (M-CSF), RANKL, osteoprotegerin (OPG) and TNF-α initiates the resorption of this mineralized cartilage.
- During this process M-CSF, RANKL and OPG are also thought to help recruit bone cells and osteoclasts to form woven bone.
- TNF-α further promotes the recruitment MSC with osteogenic potnetial
- Initiates chondrocyte apoptosis.

Healing phase

- Mitochondria accumulate calcium-containing granules created in the hypoxic fracture environment.
- After elaboration into the cytoplasm of fracture callus chondrocytes, calcium granules are transported into the extracellular matrix where they precipitate with phosphate and form initial mineral deposits.
- These deposits of calcium and phosphate become the nidus for homogeneous nucleation and the formation of apatite crystals.
- The peak of the hard callus formation is usually reached by day 14

Remodeling

- The remodeling process is carried out by a balance of hard callus resorption by osteoclasts, and lamellar bone deposition by osteoblasts.
- Although the process is initiated as early as 3–4 weeks in the remodeling may take years to be completed to achieve a fully regenerated bone

Remodeling

- Bone remodeling has been shown to be a result of production of electrical polarity created when pressure is applied in a crystalline environment.
- This is achieved when axial loading of long bones occurs, creating one electropositive convex surface, and one electronegative concave surface, activating osteoclastic and osteoblastic activity respectively.
- By these actions the external callus is gradually replaced by a lamellar bone structure, whereas the internal callus remodelling re-establishes a medullar cavity characteristic of a diaphyseal bone.