

The end of the inflammatory process:

Tissue regeneration: Healing or fibrous repair

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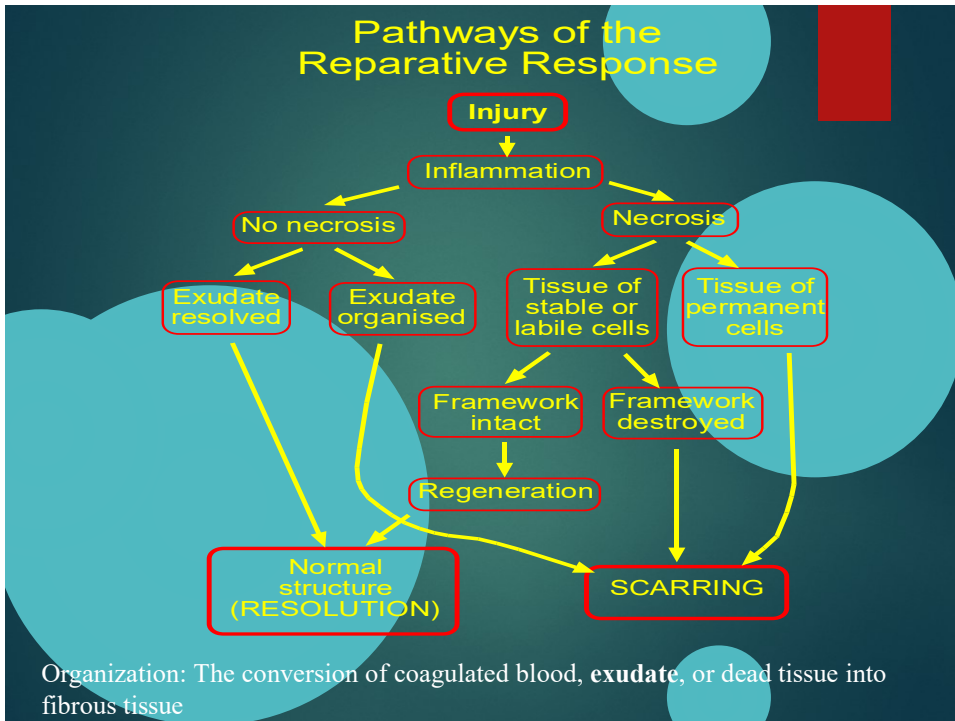
Wound Healing Process

Tissue damage elicits acute inflammation and demolition

The wound healing process leads to a restoration of the integrity and function of the tissue:

- migration and proliferation of parenchymal and connective tissue cells
- synthesis of extra cellular matrix (ECM) proteins, by fibroblasts and other cells
- regeneration of parenchymal cells into functional tissue
- remodeling of connective tissue and parenchymal cell components
- collagenization and development of wound tensile strength

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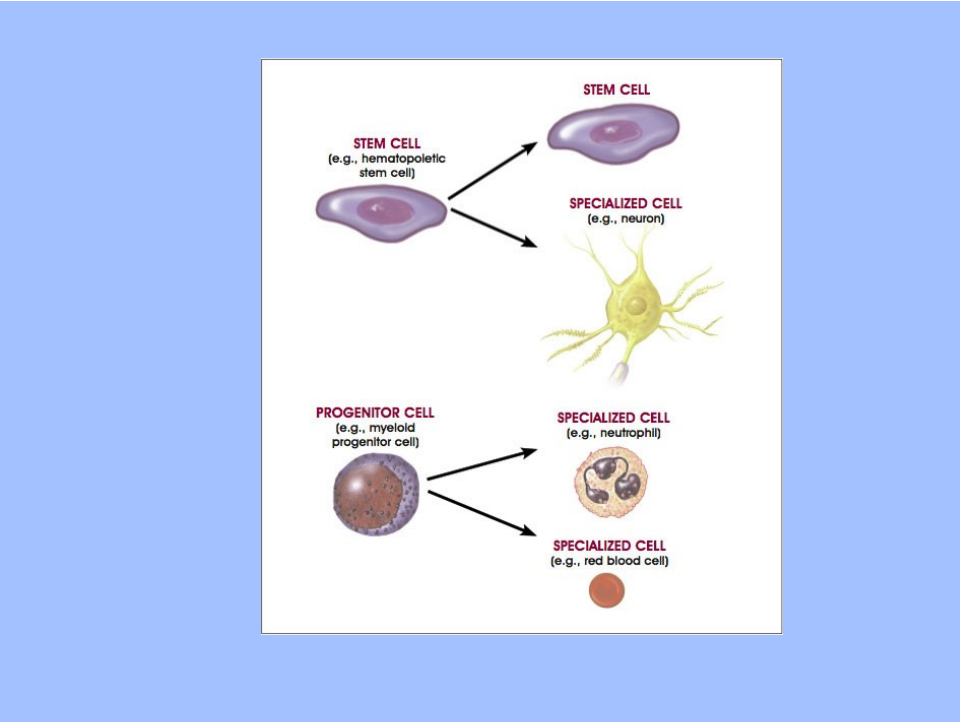
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The ability of a tissue to regenerate itself is dependent on the presence of :

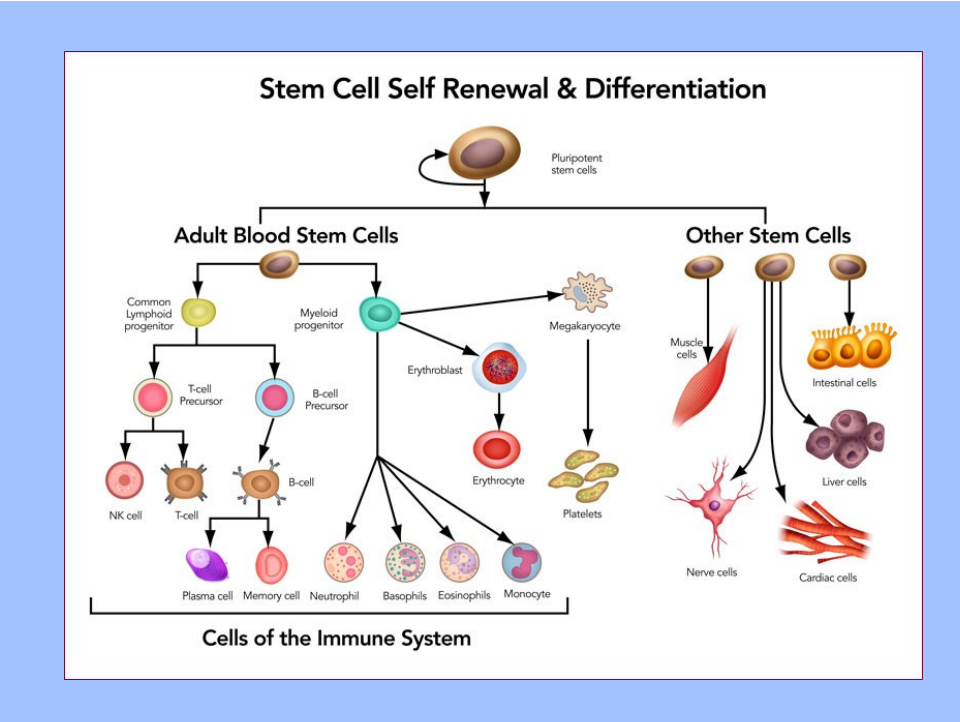
Stem cells (in particular: Adult Stem cells), which are cells:

- Able to renew themselves (symmetric division)
- Able to generate a progeny of cells that may differentiate into different tissue (asymmetric division)

4



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6

REGENERATION

- **Labile cells:**
 - normal state is active cell division
 - usually rapid regeneration
 - These tissues contain pools of stem cells
- **Stable cells:**
 - not normally dividing at a significant rate
 - may enter the cell cycle in response to certain stimuli, such as cell injury
 - speed of regeneration variable
- **Permanent cells:**
 - Have left the cycle permanently
 - unable to divide
 - No regeneration: replacement

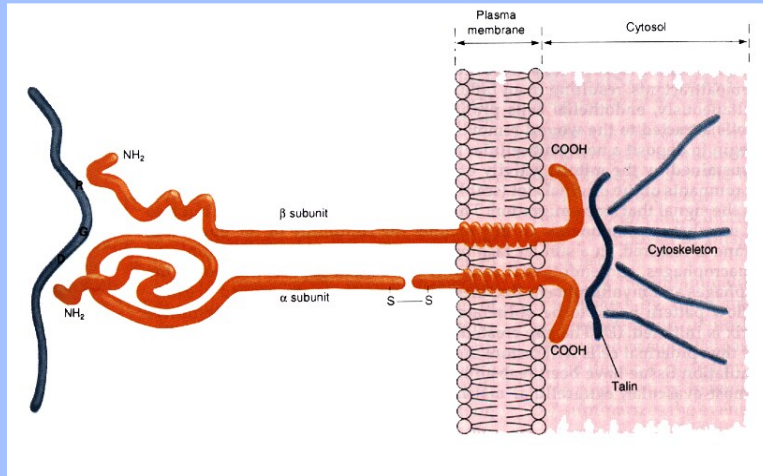
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Factors controlling regeneration:

- Complex and poorly understood.
- 'Growth factors':
 - EGF, PDGF, FGF, IGF ...
 - Hormones e.g. ACTH, estrogen, growth hormone...
 - Contact with basement membranes & adjacent cells
 - Signalling through **integrins**

8

Integrins



9

Major steps in wound healing and their associated growth factors

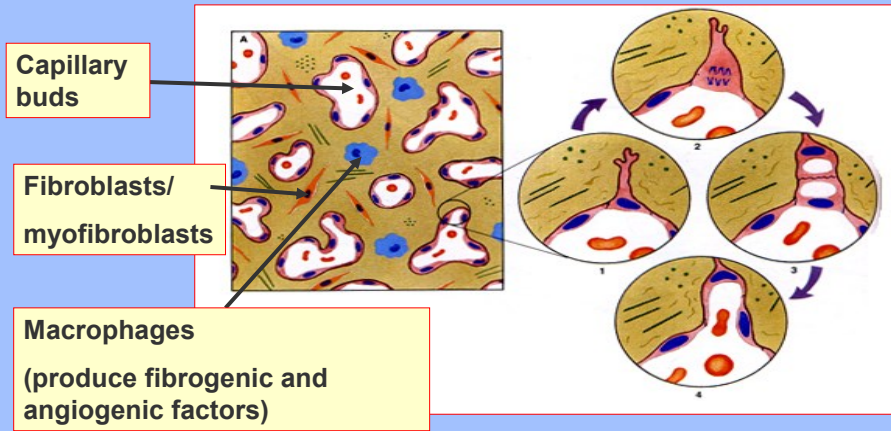
	EGF	FGF	KGF	PDGF	TGF- α	TGF- β	TNF	VEGF
Fibroblast migration		X		X		X		
Fibroblast proliferation	X	X		X	X		X	
Monocyte migration		X		X		X	X	
Macrophage activation							X	
Epithelial migration	X	X	X		X			
Epithelial proliferation	X	X	X		X			
Angiogenesis		X		X	X		X	X
Collagen synthesis				X		X		
Collagenase synthesis	X	X		X			X	X
Wound contraction		X		X				

EGF, epidermal growth factor; FGF, fibroblast growth factor; KGF, keratinocyte growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

10

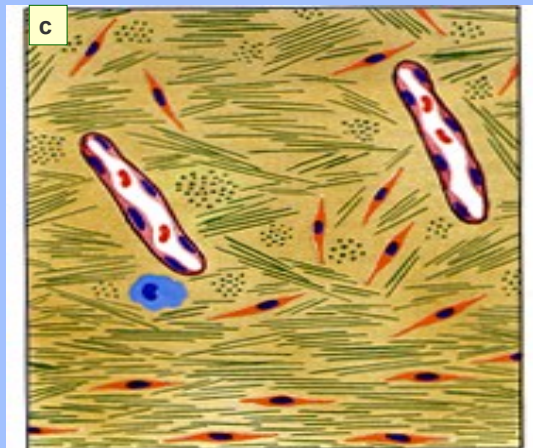
Organization and repair

- a) Debris removal by macrophages
- b) Vascular granulation tissue



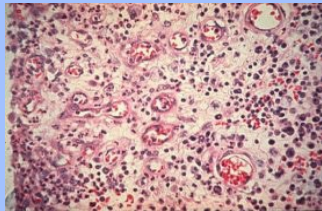
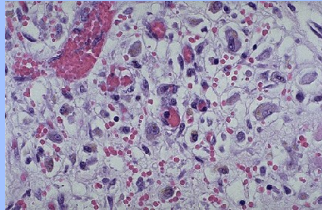
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- c) Vascular granulation tissue
(Fibroblasts proliferate and deposit collagen fibers)



12

Granulation Tissue



The initial repair response to a wound, consists of:

- richly vascular connective tissue (**capillary sprouts**, leaky new blood vessels)
- collagen
- reactive **fibroblasts** and myofibroblasts,
- variable numbers of inflammatory cells (especially **macrophage**)
- enzymes and mediators.

13

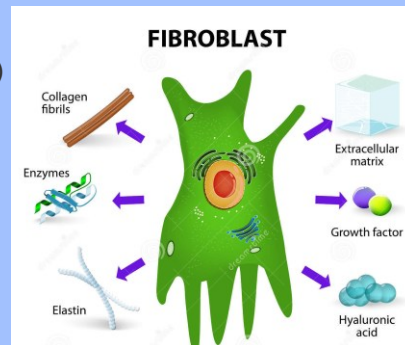
Fibroblasts

- Migration and proliferation of fibroblasts are stimulated by growth factors:

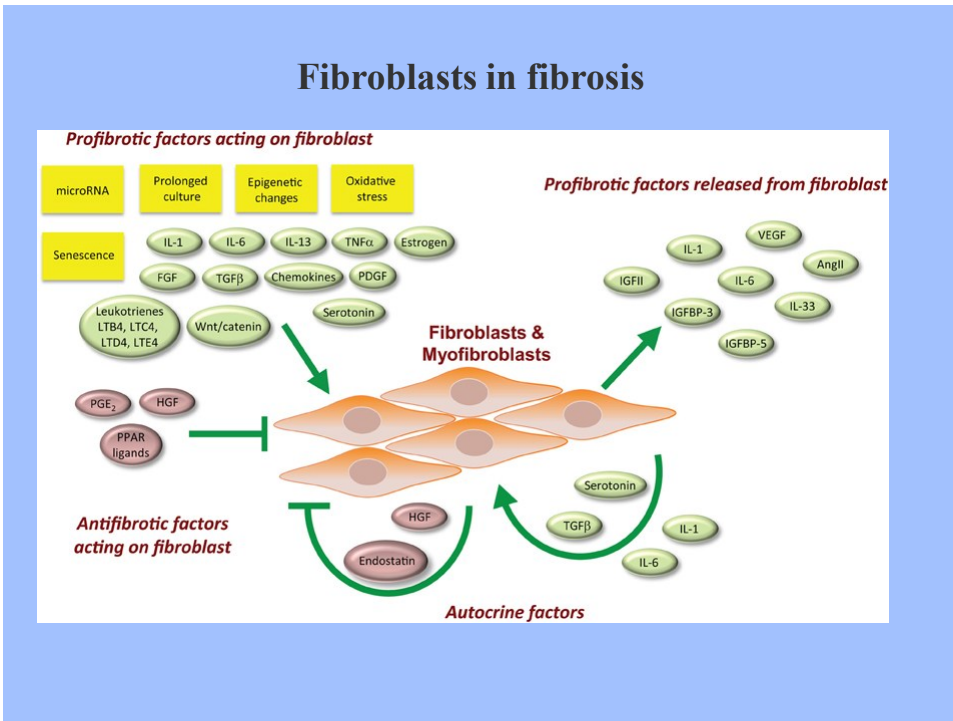
- PDGF (platelet derived growth factor)
- EGF (epidermal growth factor)
- FGF (fibroblast growth factor)

- TGF-B (transforming g. f. beta):

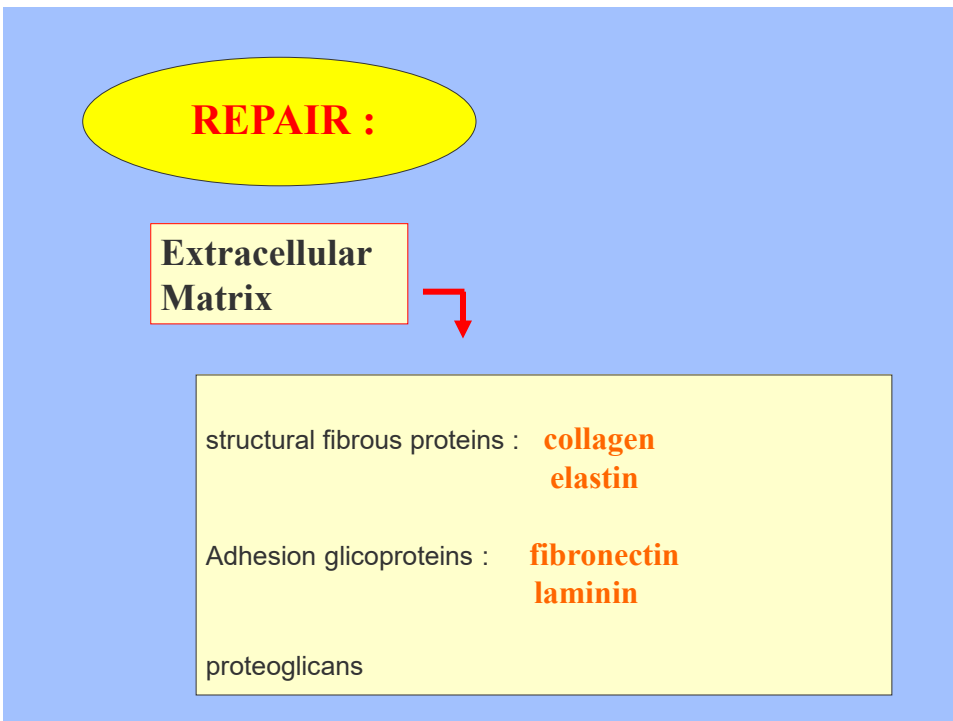
- Monocyte chemotaxis
- Fibroblast migration and proliferation
- Increases synthesis of **collagen**, **ECM**, and **fibronectin**
- decreases degradation of collagen and ECM
- Growth inhibitor for most epithelial cell types



14

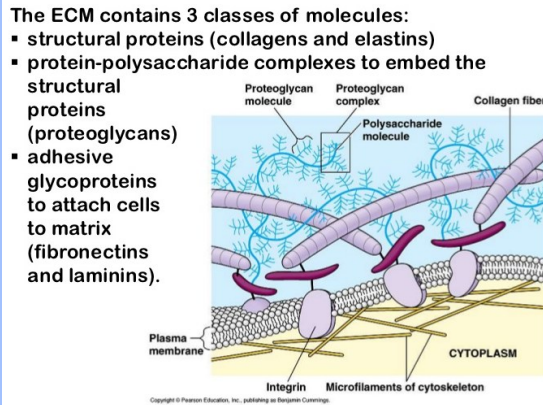


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16

The ExtraCellular Matrix

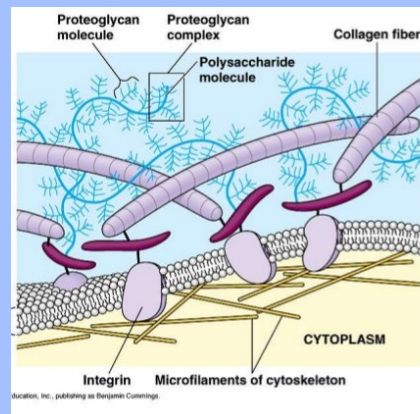


- Stable **complex of macromolecules** underlying epithelial cells and surrounding connective tissue cells.
- Provides a **structure** into which replacement cells can come for wound resolution, creating order and tissue strength.
- Migrating cells attach to it.
- A special form of ECM forms basement membranes.

17

Extracellular Matrix (ECM) Composition

- **collagens** (10 types, vary in location):
 - Type I - Bone, tendon, scars.
 - Type III - 'tissue scaffold'.
 - Type IV - non-fibrous, basement membranes ...
- **Matrix metalloproteases** (zinc dependent) degrade and remodel collagen
- **elastin** provides recoil after stretching for skin, etc.
- Glycoproteins (Fibronectin, Osteonectin, Tenascin..)
- Proteoglycans (e.g. Heparan sulphate proteoglycan..)
- laminin



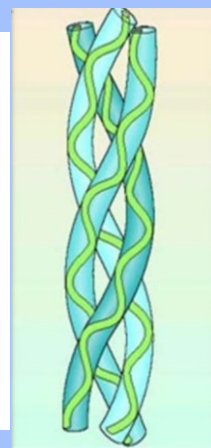
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19

Collagen structure

- All collagens are composed of 3 polypeptide alpha chains coiled around each other to form the triple helix configuration.
- The α chains are left handed helices that wrap around each other into a right handed rope like triple helical rod.
- Each such helix is around 1.4 nanometers in diameter and 300 nanometers in length
- The triple helix may be of a continuous stretch or it may be interrupted by non collagenous elements.

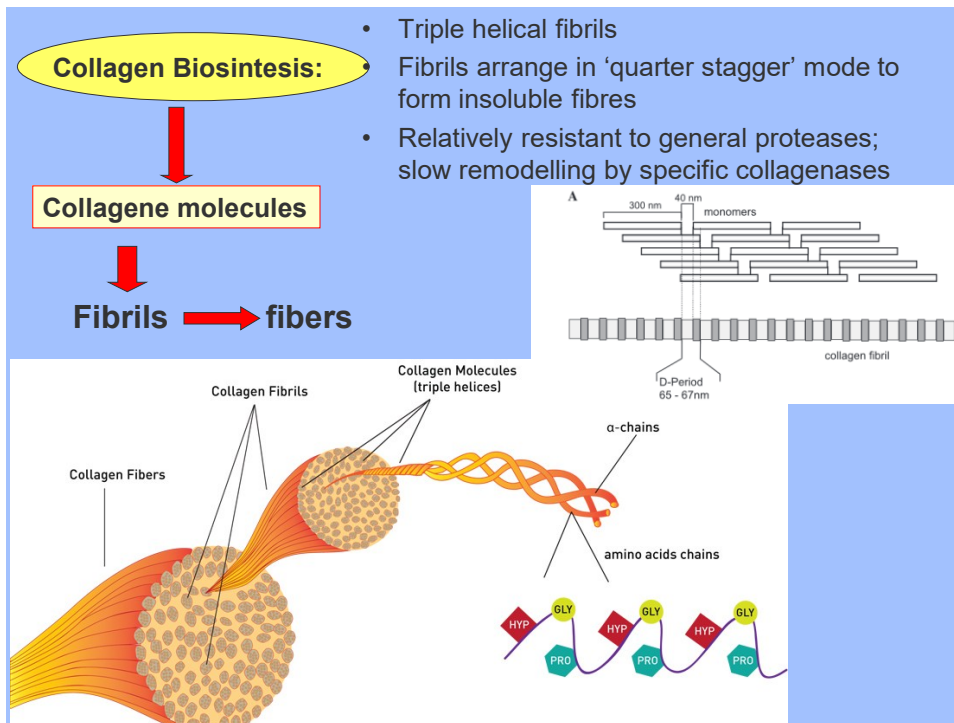


20

Collagen

1. intracellular procollagen is hydroxylated (Vit C dependent), glycosylated, secreted by fibroblasts
2. enzymatic modifications:
3. - terminal peptide chains are removed by procollagen peptidases
4. complete fibrils/fibers/bundles form in the extracellular space
5. cross linkages between alpha chains and adjacent molecules form by oxidation rxn over weeks
6. Wounds are filled with Type 3, later replaced by Type 1
7. Remodeling requires MMP (matrix metalloproteases)

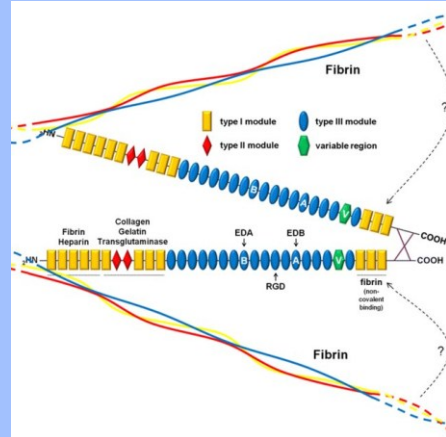
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22

Fibronectins

- Produced by fibroblasts, monocytes, and endothelial cells
- Chemotactic for fibroblasts and macrophages
- opsonin
- Organize endothelial cells into channels
- Cross links with fibrin, fibrinogen and collagen to increase tensile strength of collagen
- Binds to ECM components via integrin receptors
- Directly involved in attachment, spreading, and migration of cells



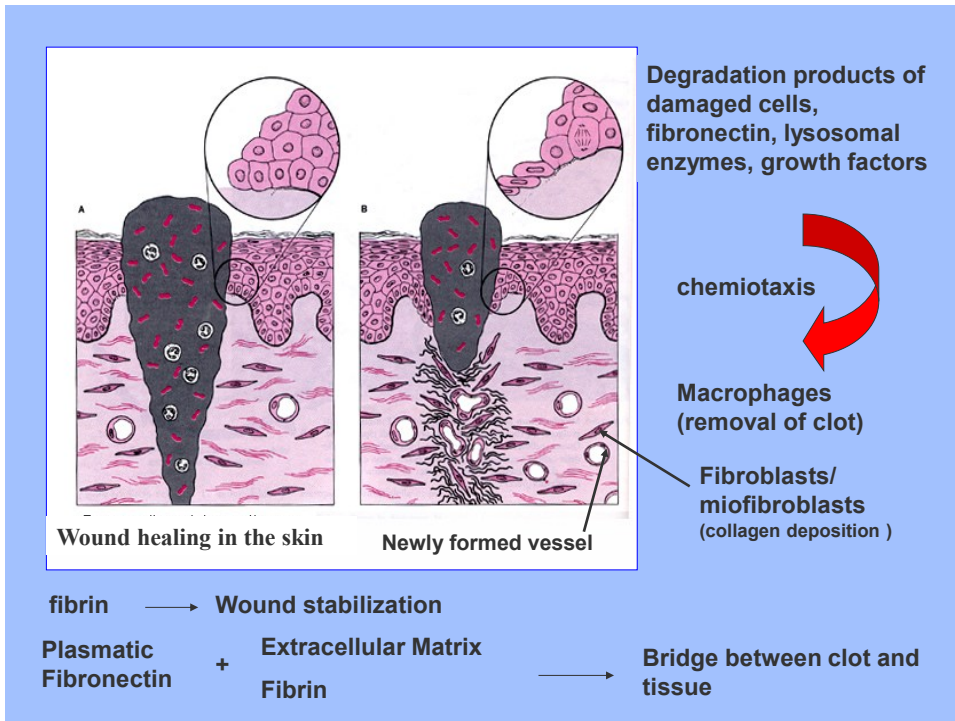
23

Wound Healing

Proliferative phase occurs after the neutrophils have removed cellular debris and release further cytokines acting as attracting agents for macrophages.

- Fibroblasts now migrate into the wound and secrete collagen type III.
- Angiogenesis occurs by 48 hours.
- The secretion of collagen, macrophage remodeling and secretion, and angiogenesis continues for up to 3 weeks.
- The greatest increase in wound strength occurs during this phase.

24



25

Angiogenesis: New blood vessels

2-3 days after wounding, vascular proliferation in granulation tissue begins, lasting several days

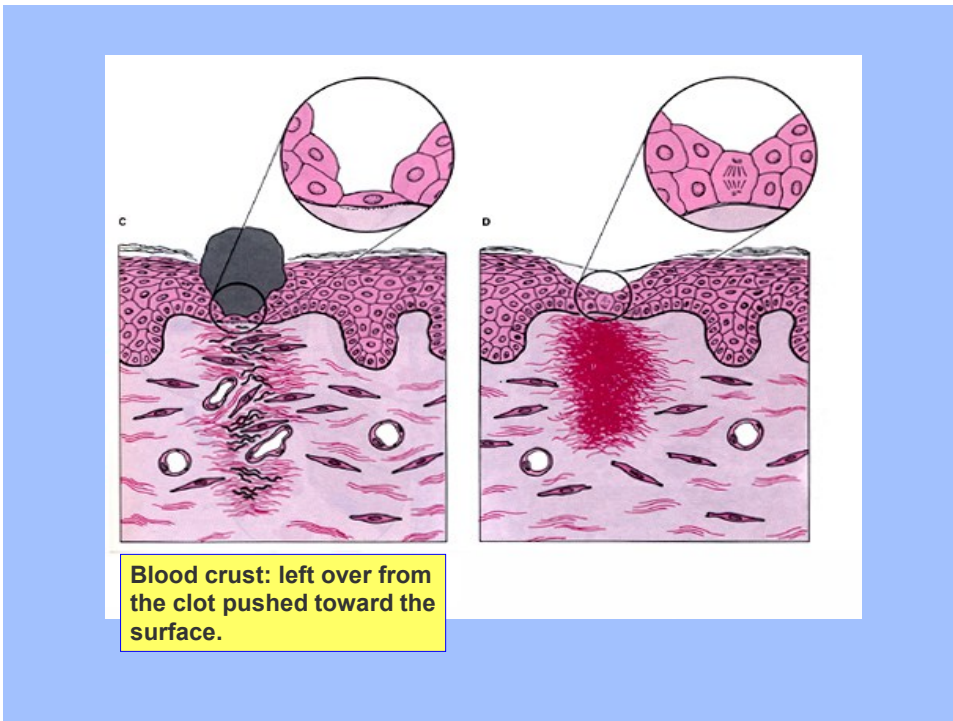
induction by

- Fibroblast Growth Factor (FGF)
- Vascular permeability factor (VPF)
- Vascular Endothelial growth factor (VEGF)

phases in the process

- Proteolytic degradation of the basement membrane allowing for formation of a capillary sprout
- Migration of endothelial cells toward an angiogenic stimulus
- Proliferation of endothelial cells
- Maturation of endothelial cells and organization into capillary tubes

26



27

Types of Cells and Repair potential

- Labile cells
 - replaceable from stem cells
 - e.g., squamous epithelium of skin, mucous membranes, bone marrow
- Stable cells
 - replacement requires forcing G0 cells to cycle
 - e.g. kidney tubule cells, hepatocytes, smooth muscle cells
- Permanent cells
 - nondividing, replaced by a scar or a cavity
 - e.g. brain

28

SKIN WOUNDS

Classification of Wounds Closure

- **Healing by Primary Intention:**
 - All Layers are closed. minimal scar formation.
- **Healing by Secondary Intention:**
 - Deep layers are closed but superficial layers are left to heal from the inside out.
 - Healing by second is appropriate in cases of infection, excessive trauma, tissue loss, or imprecise approximation of tissue.

29

- **Healing by Tertiary Intention:**

Also referred to as **delayed primary closure**.

in which the wound is left open for a number of days and then closed if it is found to be clean.

This technique is used in badly contaminated wounds to allow drainage and thus avoid the entrapment of microorganisms.

30

Healing by “Primary Intention”

All Layers are closed. The incision that heals by first intention does so in a minimum amount of time, with no separation of the wound edges, and with minimal scar formation.

- Epidermis regenerates
- Dermis undergoes fibrous repair.
- Sutures out at 5-10 days: approx. 10% normal strength.
- Maturation of scar continues up to 2 years.
- Minimal scarring, good strength
- Risk of trapping infection under skin - produces abscess.

31

Healing by "primary" or "first intention"

Early events during Days 1-3:

- Wound occurs: hemorrhage, clotting, cell injury, minor acute inflammation
- Neutrophils appear at margins.
- Mitotic epithelial cells at margins migrate across the wound
- Macrophages infiltrate the defect and break down fibrin. Thin surface epithelial layer forms by cell migration and fusion.
- Surface layer is thickening. Macrophage predominate.
- Vertical collagen fibrils appear along the margins.
- Granulation tissue progressively enters the tissue space

32

Wound Healing: Week 1 and 2

Day 5: Wound space is filled with granulation tissue, collagen fibrils begin to bridge the site, surface epithelium is normal thickness and architecture, acute inflammation is regressing.

Day 7: Sutures are removed. Wound has 10% of tensile strength of normal skin.

Day 7-15: More fibroblast proliferation and collagen deposition follows the lines of tissue stress with an increase in wound strength; Leukocytic infiltrate, edema, increased vascularity decreases.

33

Wound Healing: Final Stages

Day 30: Wound now is devoid of infiltrate, largely covered by intact normal epidermis; 50% strength, Type III collagen is slowly being replaced by Type I via action of collagenases and MMPs

3 Months: Wound is 80% of normal. Complete blanching of a scar takes longer

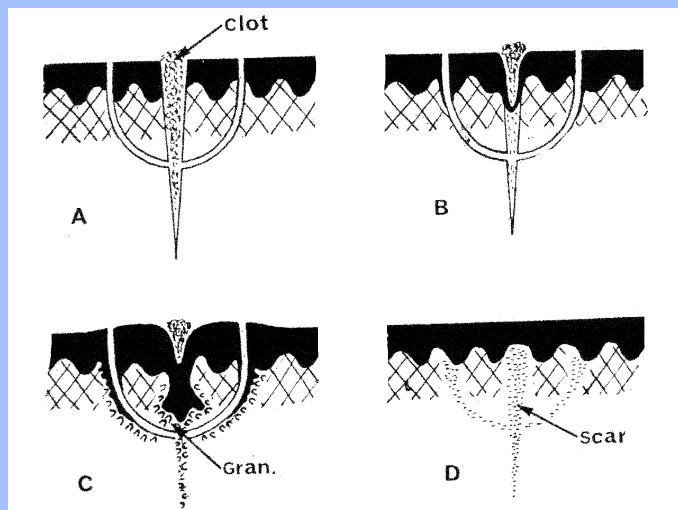
34

Wound Healing

- **Maturation phase** is the final phase and starts from the 3rd week and continues for up to 9-12 months.
- This is where collagen III is converted to collagen I, and the tensile strength continues to increase up to 80% of normal tissue.

35

Healing by 'primary intention': A clean, sutured wound.



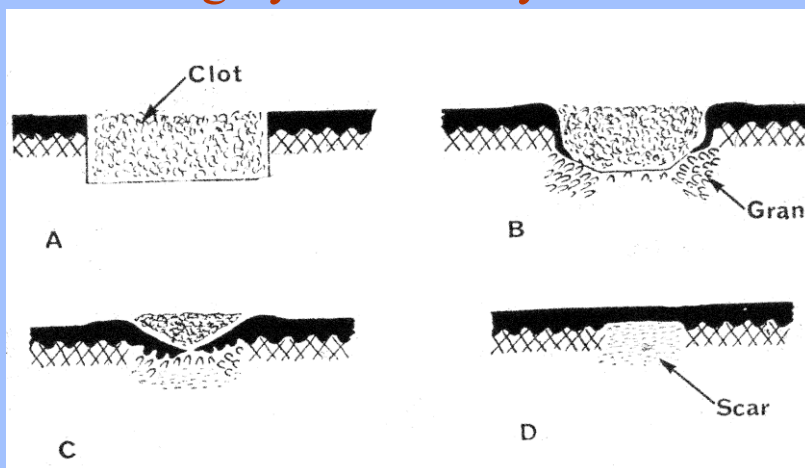
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SKIN WOUNDS: Healing by “Secondary Intention”

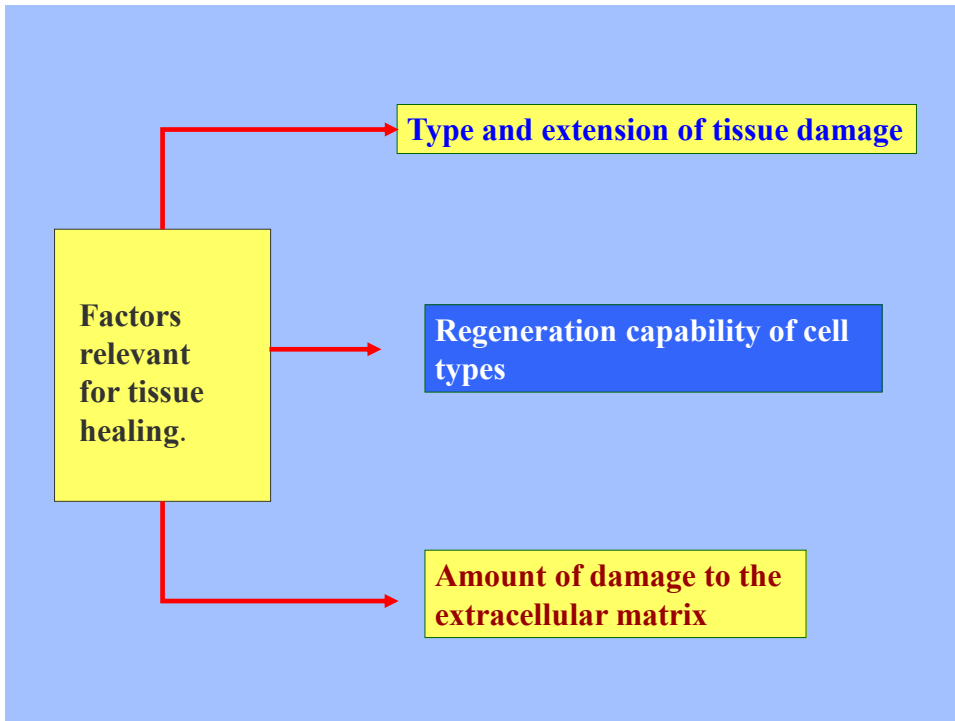
- Quantitative differences.
 - Initial contraction (in furry animals!)
 - Clot dries to form a ‘scab’ or ESCHAR
 - Epidermis regenerates beneath.
 - Repair process produces GRANULATION TISSUE
- Comparison with primary intention:
 - Takes longer
 - Produces a larger scar; not necessarily weaker
 - Produces more late contraction

37

An open wound: Healing by ‘secondary intention’



38



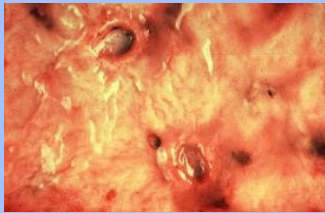
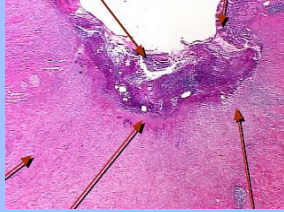
39

Issues in Successful Resolution

- extent of tissue necrosis
 - Support stroma must be intact
- type of parenchymal cells involved
 - cells must be able to regenerate
- adequacy of demolition
 - debris and fibrin impair healing, induce scarring
- arterial perfusion and venous drainage
 - e.g., scaphoid bone (hand) has poor circulation
- approximation- rough or tight
 - Hint: evert wound edges

40

An Ulcer



- This slide shows in lining of the stomach with two punched out areas
- An erosion which extends down past the muscularis mucosa is an ulcer

41

Occurrence of Larger Deficits

More extensive tissue loss means a loss of ECM upon which to pattern tissue architecture

Objective of the response:

- seal the surface, and when possible, fill the hole to preserve overall structure

Examples:

- e.g. ragged wound edges, ulcer or an infarct
- permanent parenchymal cells which cannot divide

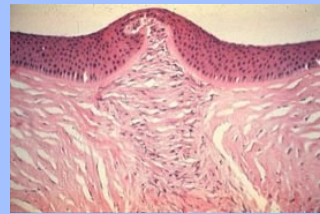
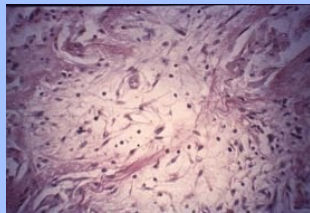
42

How is 'secondary union' different than primary?

- large gaps have more fibrin and more necrotic debris, thus more intense inflammatory reactions
- more granulation tissue occurs and thus more scarring
- patent wound contraction is possible, esp. in skin (to 90%) (myofibroblasts)
- result is healing by "secondary union, secondary intention"
- progression into chronic inflammation is possible

43

Scars



44

Physiological impairments to normal healing

Physiological impairments weakening the immune system and healing:

- malnutrition or specific deficiency of Vitamin C, Copper, or zinc
- tissue hypoxia
- leukopenia (monocytes or neutrophils)
- Fibrinogen deficiency
- Diabetes
- severe anemia
- hormonal imbalances and various syndromes

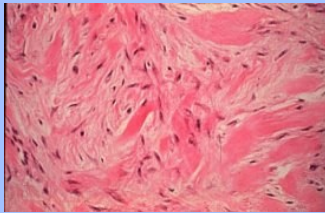
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Other Interferences to Normal Healing

- **Infection** (most common), or **foreign bodies** can lead to "chronic inflammation"
- **Excessive contractures**, e.g. burns
- **Genetic and idiopathic predispositions** lead to improper healing
 - **Keloid**: genetically predisposed production of hypertrophic scar tissue (type III collagen).
 - **exuberant granulation** tissue formation (Proud flesh) protrudes above the skin and blocks re-epithelialization
 - **Desmoids** (aggressive fibromatosis) reoccurring proliferation of fibroblasts follows incision of scars or traumatic injuries

46

Keloid



- hypertrophic scarring
- an over abundant deposition of Type III collagen
- Influenced by genetic predisposition (is more common in blacks)

47

Healing in various tissues :

Liver: We can have complete parenchymal regeneration and/or fibrosis/scar

Biopsy: cicatrization

focal Necrosis: complete regeneration.

massive Necrosis: lethal in few days, if survives the acute phase, residual hepatocytes form irregular regeneration regions (nodules) separated by large scars (cirrhosis)

Kidney : limited regeneration capability:

**high regeneration in cortical tubules,
minimal in medullar tubules ,
absent in glomerula**

48

Lung:

The lining epithelium has ample capacity for regeneration unless it has been damaged the underlying extracellular matrix. If the damage involves Basal Membrane, repair occurs by scarring and fibrosis.

Heart :

Myocardial cells have not regenerative capacity; Tissue damages heal by deposition of granulation tissue and scarring.



49

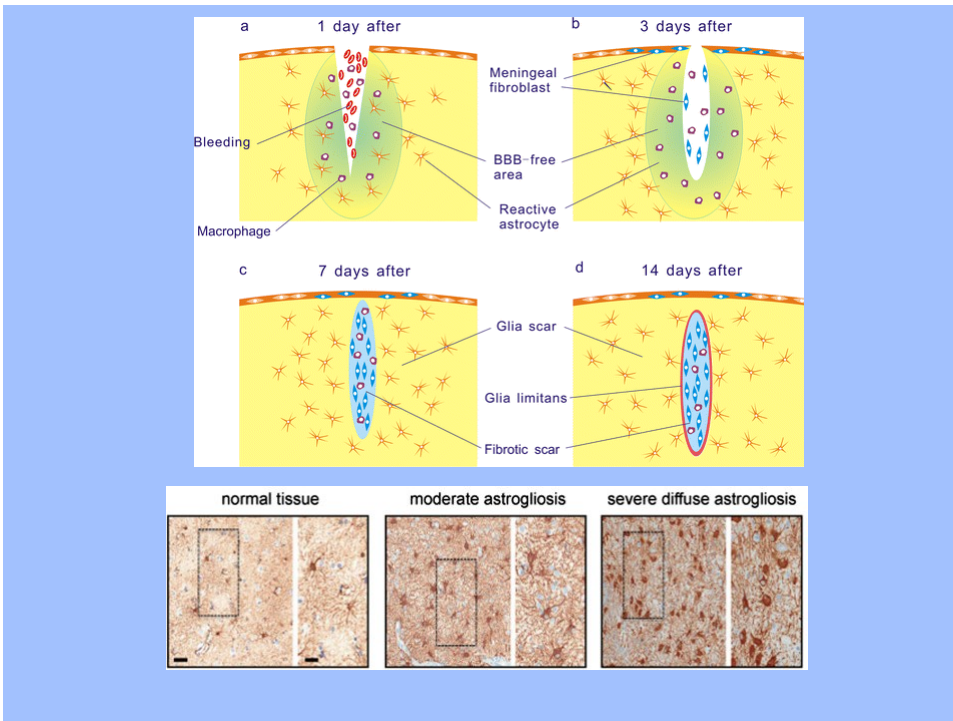
CNS :

Damage to the brain or spine leads to the proliferation of astrocytes and microglia. Gliosis is equivalent to scarring in other tissues.

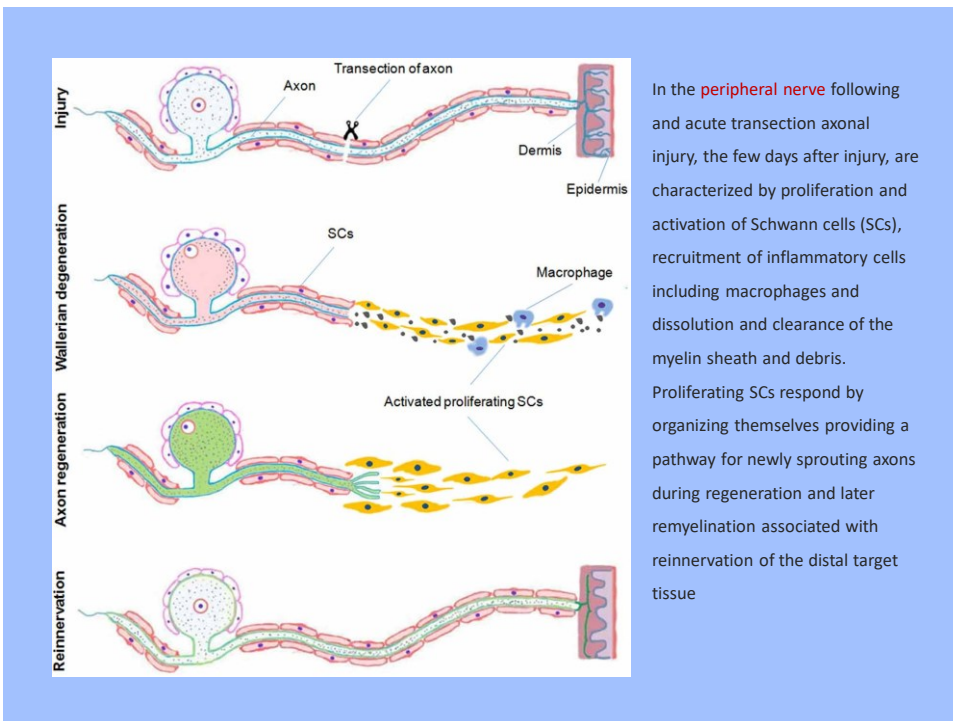
PNS :

Axonal regeneration is up to 2 weeks from the injury if there is alignment of the extremities.

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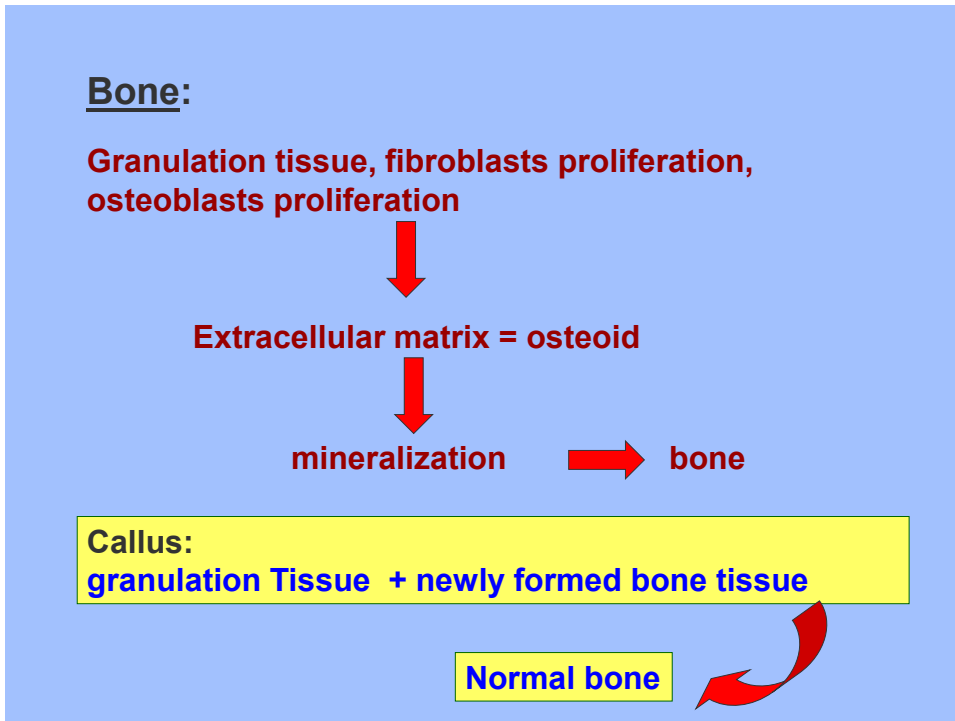


51



In the **peripheral nerve** following and acute transection axonal injury, the few days after injury, are characterized by proliferation and activation of Schwann cells (SCs), recruitment of inflammatory cells including macrophages and dissolution and clearance of the myelin sheath and debris. Proliferating SCs respond by organizing themselves providing a pathway for newly sprouting axons during regeneration and later remyelination associated with reinnervation of the distal target tissue

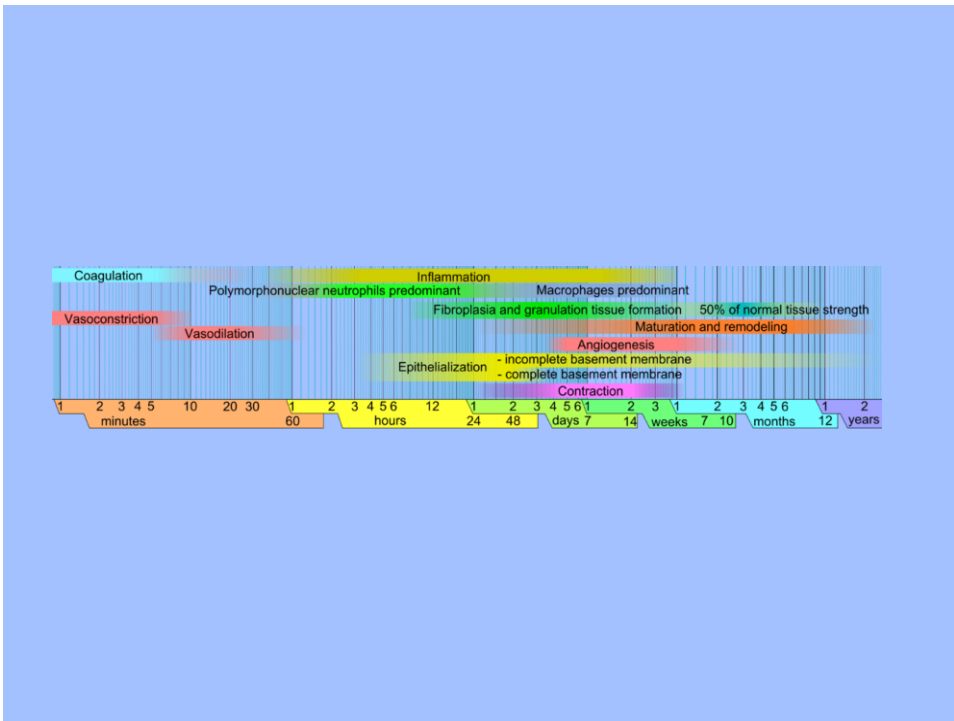
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53

Sum-up: Regeneration and Fibrous Repair/Replacement

54

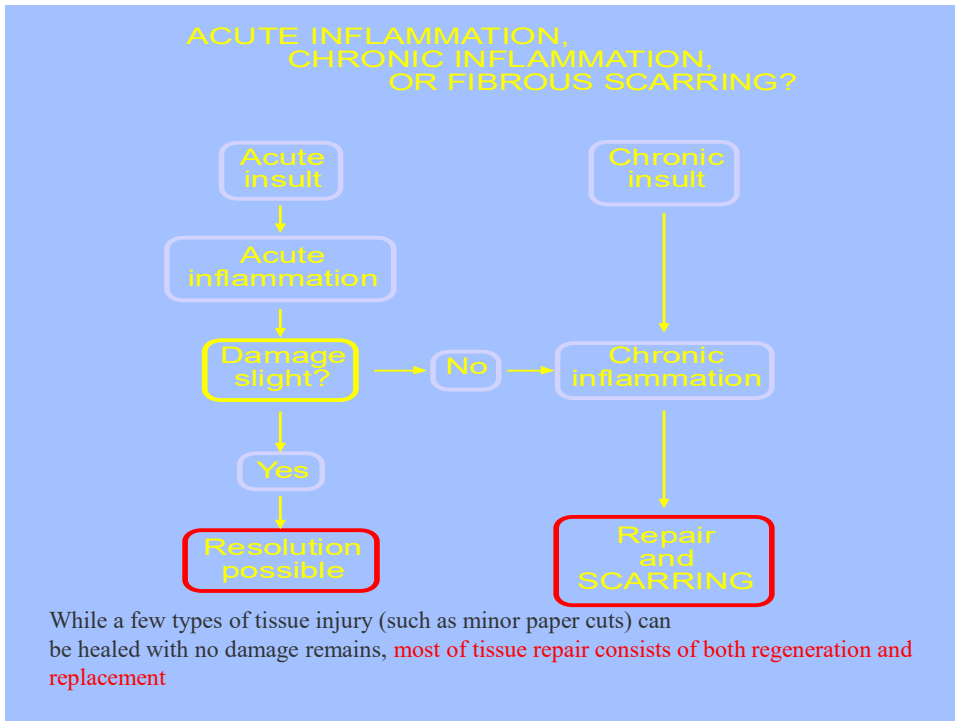


55

Tissue Repair: REGENERATION AND REPLACEMENT

- **Regeneration:**
replacement of functional, differentiated cells: new growth completely **restores** portions of damaged tissue to their normal state
- **Replacement:**
production of a fibrous scar: severely damaged or non-regenerable tissues are repaired by the laying down of **connective** tissue, a process commonly referred to as “scarring”

56



57

FIBROUS REPAIR: The development of a fibrous scar.

- Rabbit ear chamber example.
 - Blood clot forms.
 - Acute inflammation around the edges.
 - Chronic inflammation: Macrophages infiltrate the clot.
 - Capillaries and lymphatics sprout and infiltrate.
 - Myofibroblasts infiltrate and differentiate.
 - Glycoproteins and Collagene are produced
 - Cell population falls, vessels differentiate and are reduced in number.
 - Collagen matures and contracts.

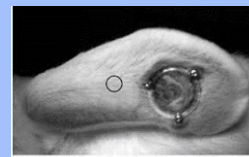
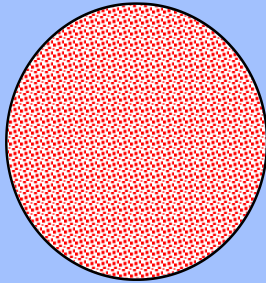


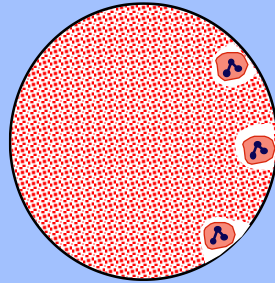
Figure 1. Rabbit ear with rabbit ear chamber (REC) and the

58

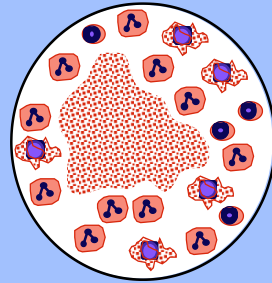
Rabbit Ear Chamber: Direct observation of fibrous repair.



1) Exudate clots.



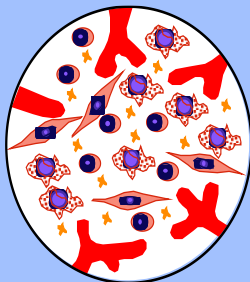
2) Neutrophils infiltrate and digest clot



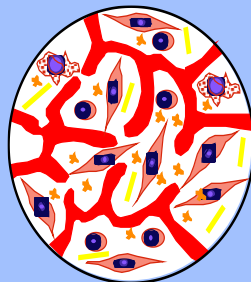
3) Macrophages and lymphocytes are recruited

59

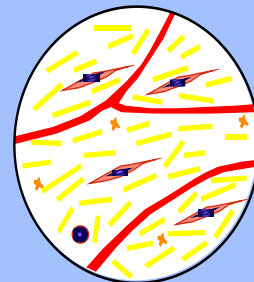
Rabbit Ear Chamber: Direct observation of fibrous repair.



4) Vessels sprout, myofibroblasts make glycoproteins

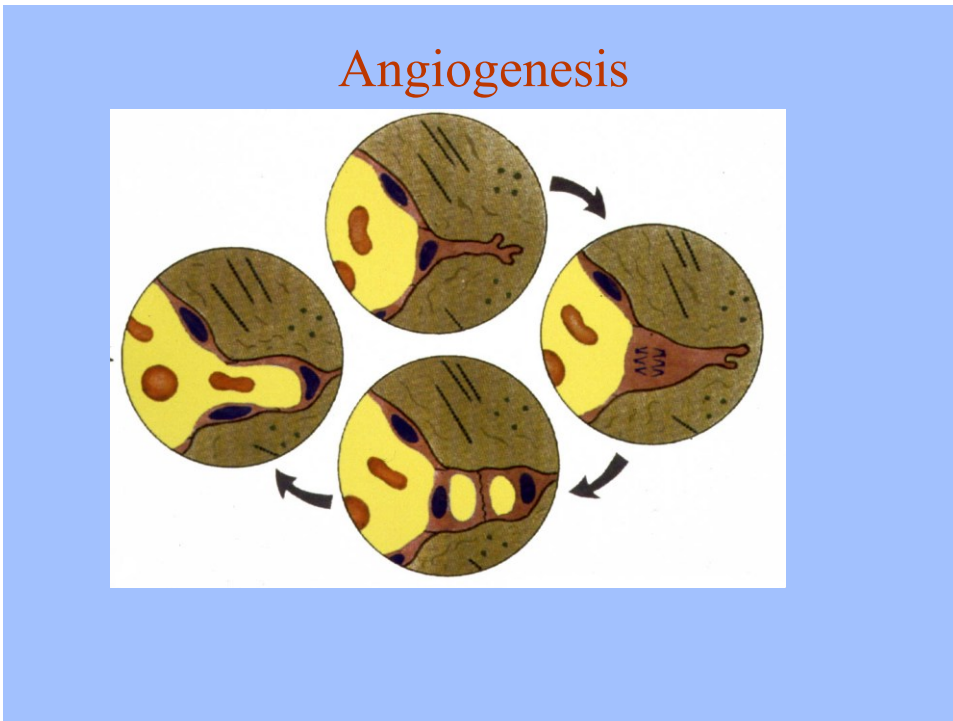


5) Vascular network; collagen synthesised; macrophages reduced



6) Maturity. Cells much reduced; collagen matures, contracts, remodels

60



61

CONTROL OF REPAIR PROCESSES

Angiogenesis

- Macrophages - Various angiogenic cytokines, e.g. VEGF, bFGF ...

- Fibrosis

- Macrophages- various pro-fibrotic cytokines, e.g. TGF beta, PDGF, ...

- Limitation of fibrosis and remodelling

- Macrophages - through the production of TGF- β , PDGF, tumor necrosis factor (TNF), osteopontin (OPN), IL-1, collagenase and matrix metalloproteinases (MMPs)

62

Major steps in wound healing and their associated growth factors

	EGF	FGF	KGF	PDGF	TGF- α	TGF- β	TNF	VEGF
Fibroblast migration		X		X		X		
Fibroblast proliferation	X	X		X	X		X	
Monocyte migration		X		X		X	X	
Macrophage activation							X	
Epithelial migration	X	X	X		X			
Epithelial proliferation	X	X	X		X			
Angiogenesis		X		X	X		X	X
Collagen synthesis				X		X		
Collagenase synthesis	X	X		X			X	X
Wound contraction		X		X				

EGF, epidermal growth factor; FGF, fibroblast growth factor; KGF, keratinocyte growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

63

FACTORS INFLUENCING WOUND HEALING (1)

- General Factors:
 - Age
 - General state of health
 - chronic diseases e.g. diabetes, rheumatoid arthritis etc.
 - Drugs (e.g. steroids) and hormones
 - General cardiovascular status
 - General dietary deficiencies e.g. protein
 - Specific dietary deficiencies
 - Vitamin C
 - sulphur-containing amino acids

64

FACTORS INFLUENCING WOUND HEALING (2)

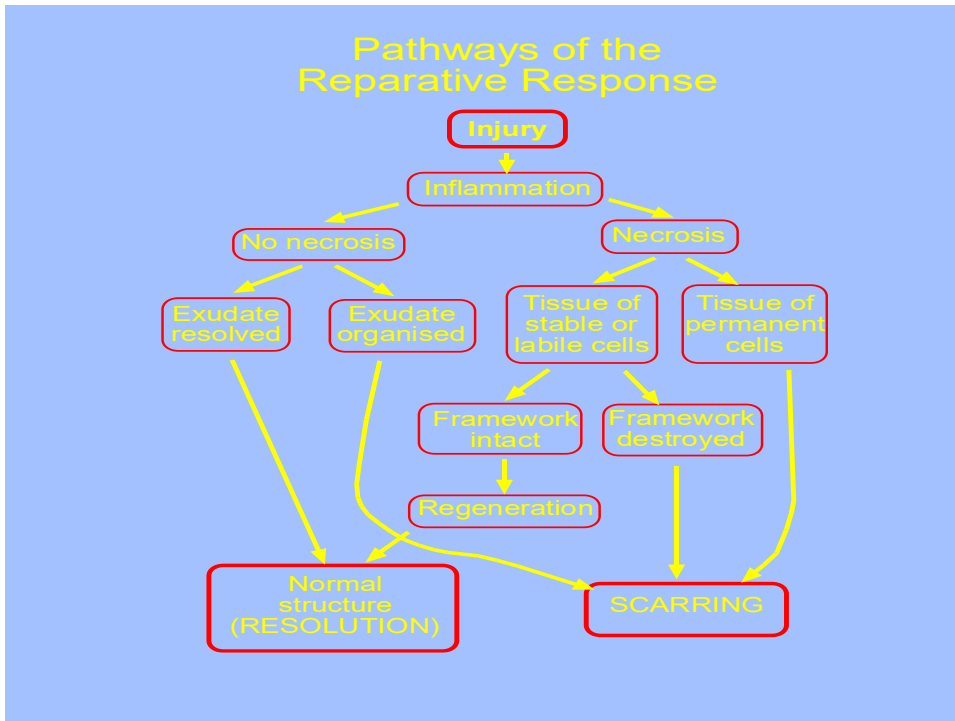
- Local factors:
 - Type, size, location of wound
 - Apposition, lack of movement
 - Infection: Suppuration, Gangrene, Tetanus
 - (Secondary hæmorrhage)
 - Blood supply: Arterial, Venous
 - Foreign material: dirt, glass, sutures, necrotic tissue
 - Radiation damage

65

Complications of Repair

- Insufficient fibrosis:
 - Wound dehiscence; hernia; ulceration
- Excessive fibrosis:
 - Cosmetic scarring; hypertrophic scars; keloid
- Excessive contraction:
 - Limitation of joint movement (Contractures);
obstruction of tubes & channels (Strictures)

66



67