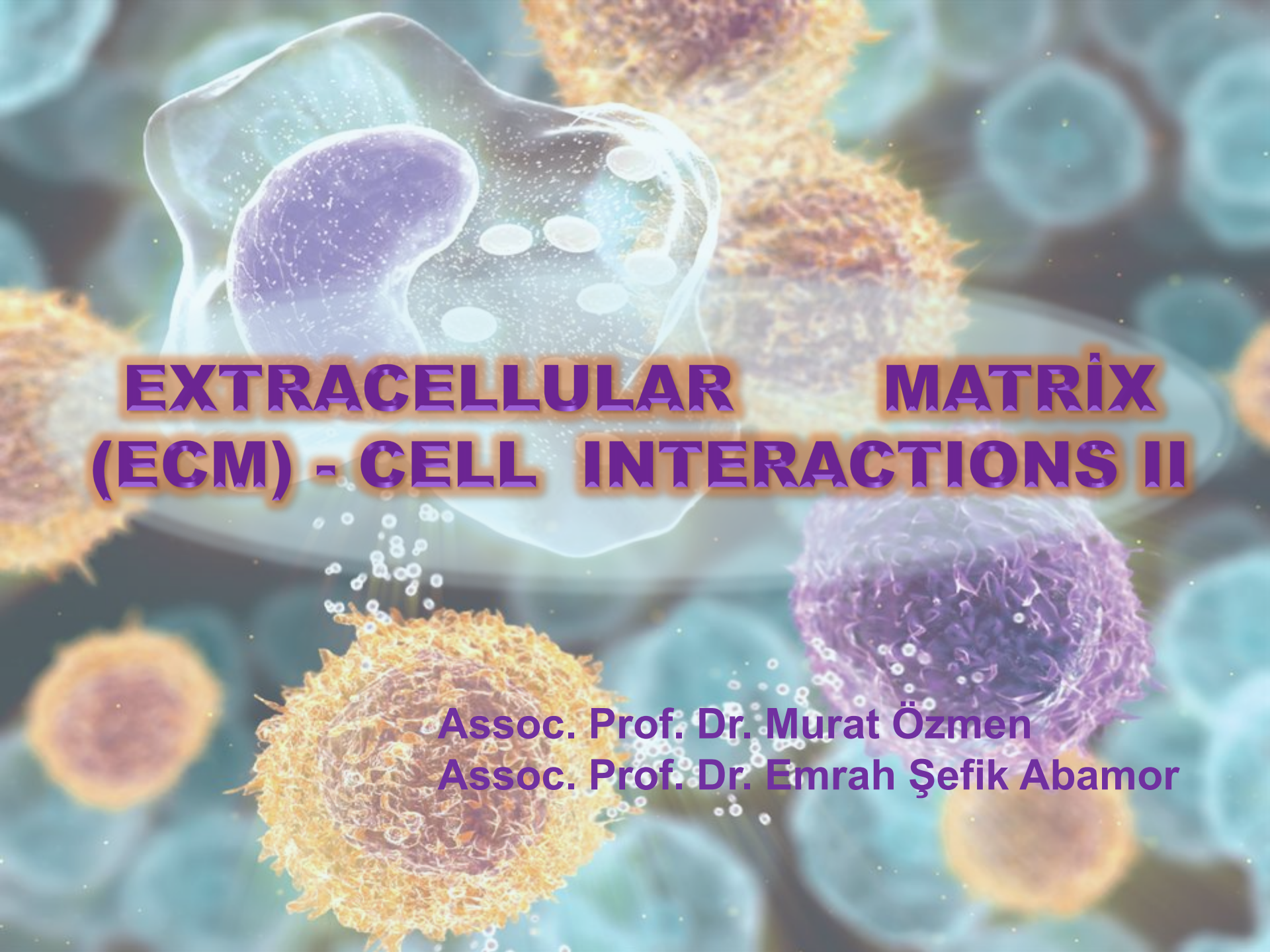


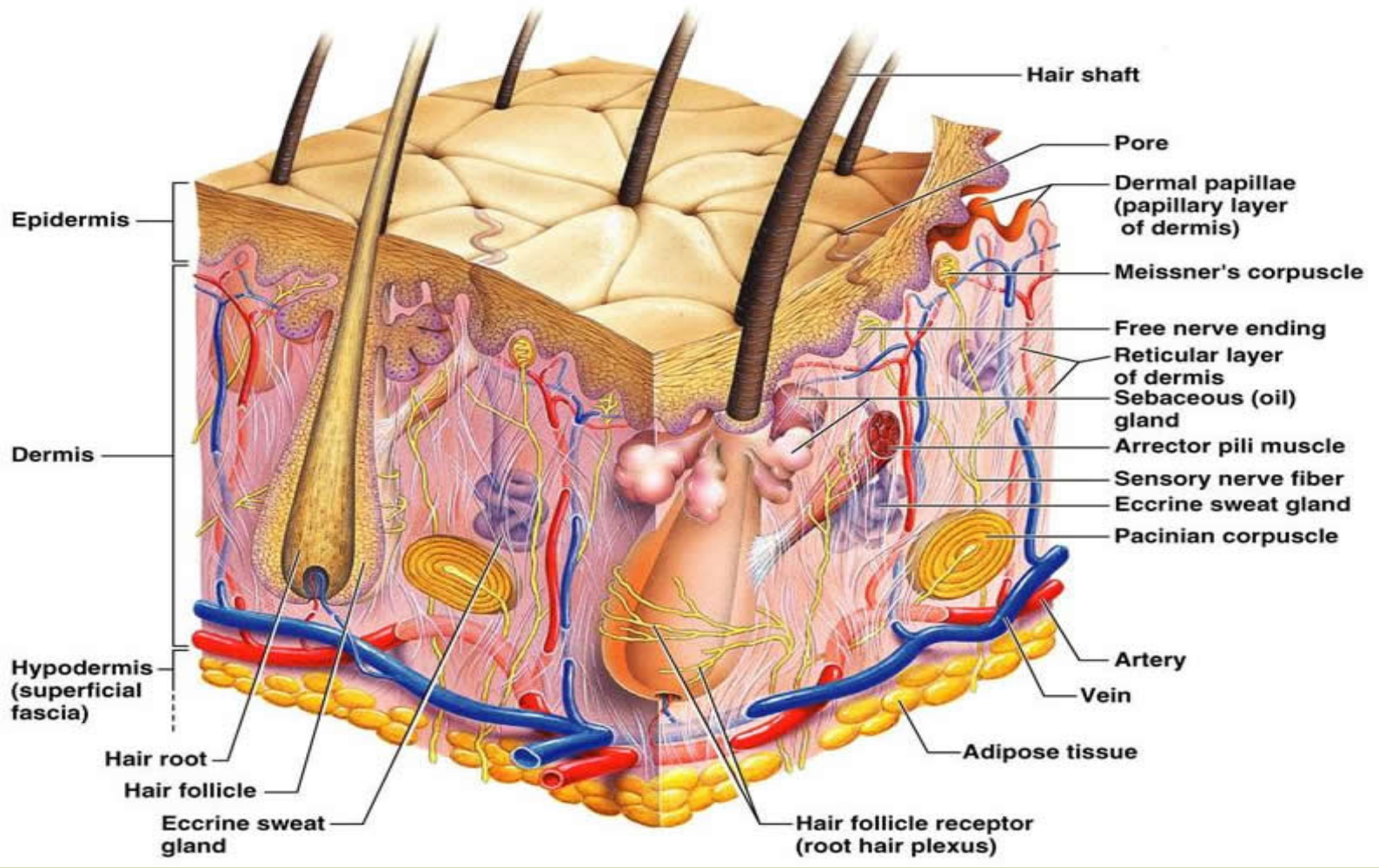
EXTRACELLULAR MATRİX (ECM) - CELL INTERACTIONS II

Assoc. Prof. Dr. Murat Özmen
Assoc. Prof. Dr. Emrah Şefik Abamor

Wound Healing

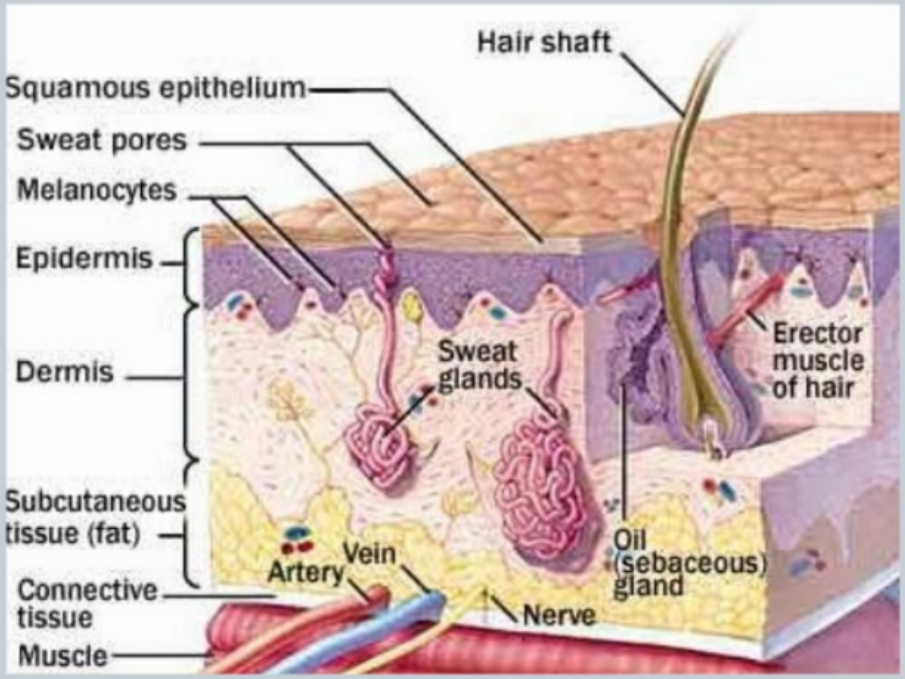
- *Wound healing is the process of repair that follows injury to the skin and other soft tissues.*
- *Healing generates resurfacing, reconstitution, and restoration of the tensile strength of injured tissue.*
- *Wound healing is a complicated biological process involving many cell types, various cytokines, growth factors and their interactions.*

The Skin Layers



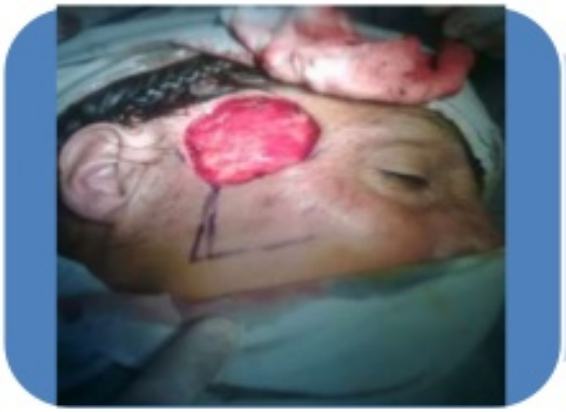
Wound classification according to depth of the wound

- Superficial
- Partial thickness
- Full thickness
- Deep wound

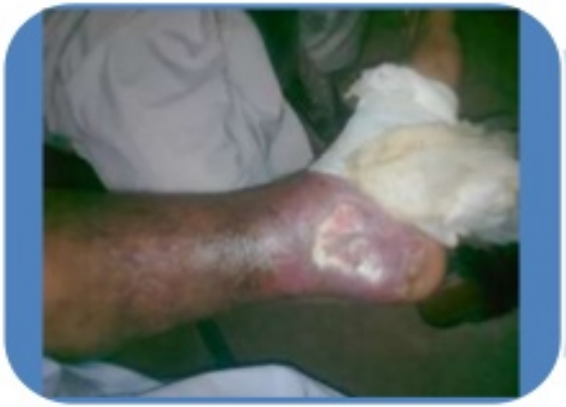


+ bone, opened cavities, organs...etc.

Wound classification according to healing duration



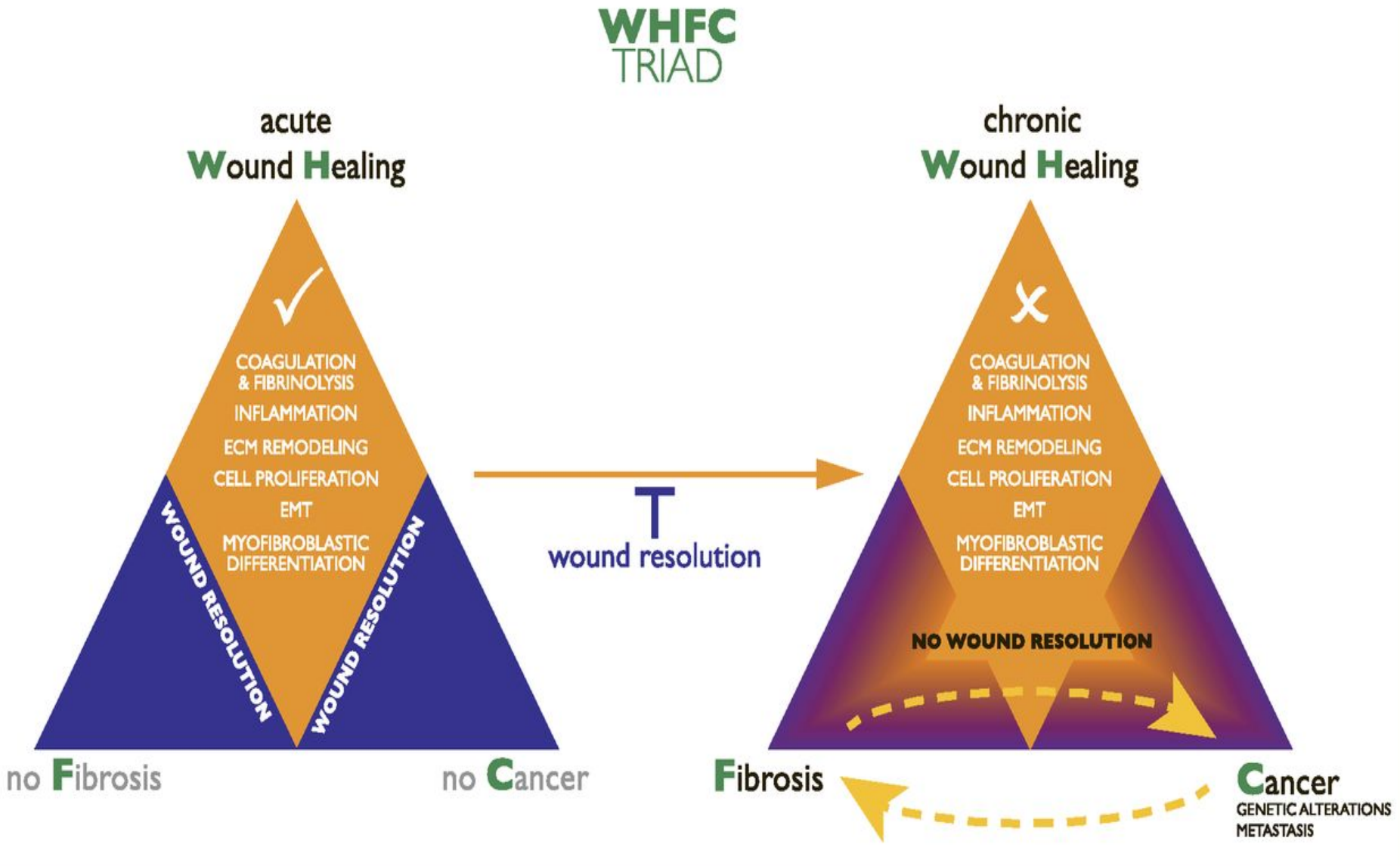
ACUTE Recent wound which has yet to progress through the sequential stages of healing



CHRONIC Wound that has arrested in one of the wound healing stages usually inflammatory phase

General differences between acute & chronic wounds

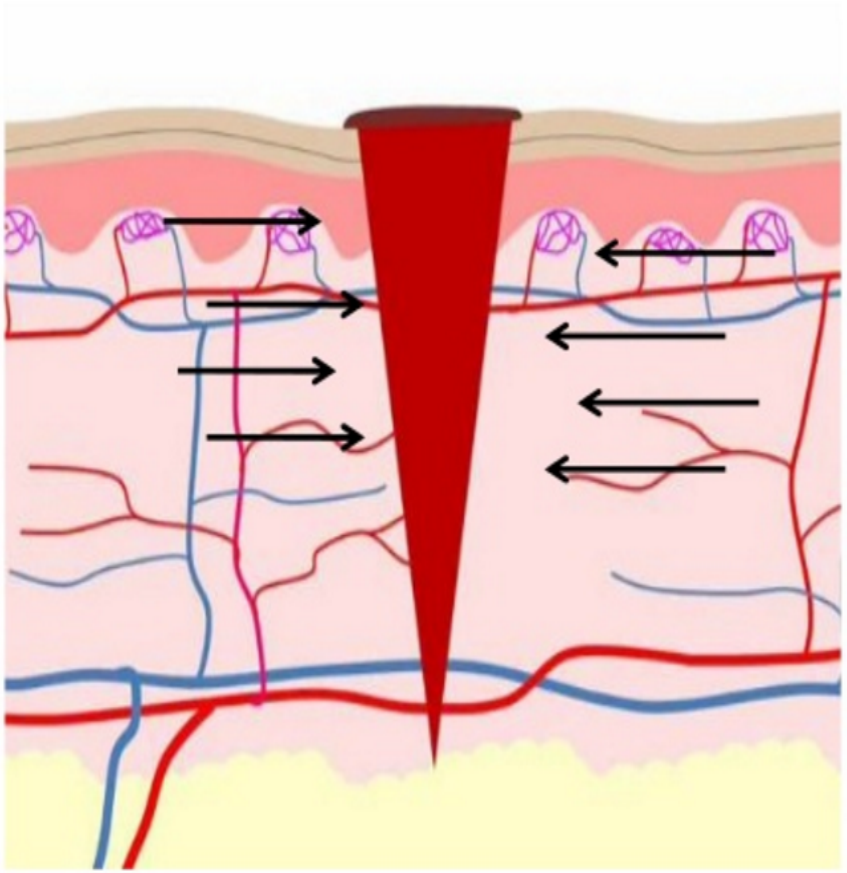
Acute	Chronic
Short duration	Not healed by 6 weeks
No underlying pathology	Underlying pathology
Normal inflammatory stage	Prolonged inflammatory stage
Usually heals without complication	A variety of complications may arise
Acute wound fluid supports proliferation	CWF does not support proliferation
Wound fluid doesn't damage peri- wound skin	CWF damaging to peri-wound skin
Neutrophil, elastase and MMP levels normal	Neutrophil, elastase and MMPs levels high
Fibrinectin intact	Fibrinectin degraded
Normal remodelling of ECM	Defective remodelling of ECM
Normal growth factor levels	Lower levels of GFs
Normal levels of inflammatory cytokines	Increased levels of pro-inflammatory cytokines


WHFC
TRIAD
acute
Wound Healing
COAGULATION & FIBRINOLYSIS
INFLAMMATION
ECM REMODELING
CELL PROLIFERATION
EMT
MYOFIBROBLASTIC DIFFERENTIATION
WOUND RESOLUTION
WOUND RESOLUTION
no Fibrosis
no Cancer
wound resolution
chronic
Wound Healing
COAGULATION & FIBRINOLYSIS
INFLAMMATION
ECM REMODELING
CELL PROLIFERATION
EMT
MYOFIBROBLASTIC DIFFERENTIATION
NO WOUND RESOLUTION
Fibrosis
Cancer
GENETIC ALTERATIONS
METASTASIS

- Types of Clinical Wound Healing
  - Primary Intention
  - Secondary Intention
  - Tertiary Intention (Delayed Primary Closure)

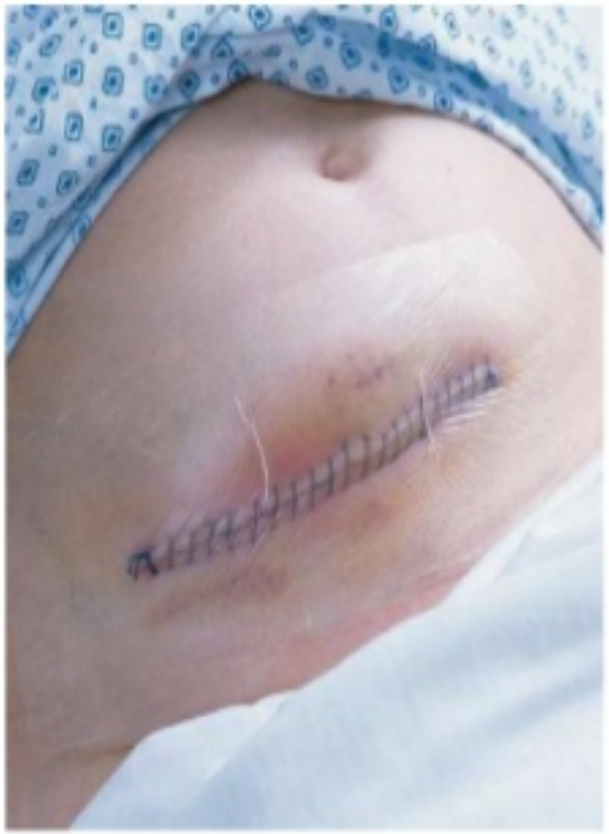
Classification of wound healing

- Primary Intention
- Occurs when:
  - The edges are clean and held together with ligatures
  - There is little gap to bridge Healing
- Healing properties (When uncomplicated)
  - Occurs quickly
  - Rapid ingrowth of wound healing cells (macrophages, fibroblasts, etc.)
  - Restoration of the gap by a small amount of scar tissue.
- soundly united within 2 weeks
- Dense scar tissue is laid down within 1 month

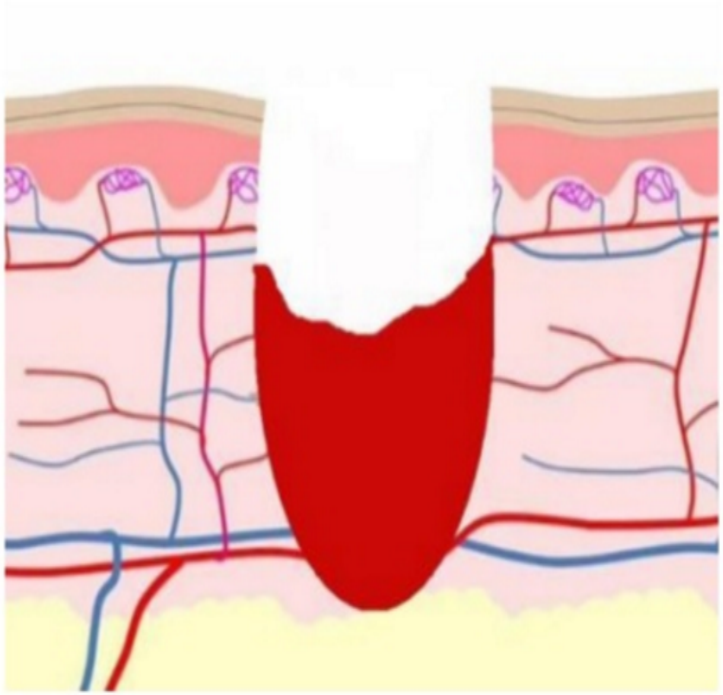


Primary Intention

- For clean wounds
- Wound is sutured/closed
- Healing occurs from side-to-side
- Healing occurs rapidly with little inflammation and minimal scarring



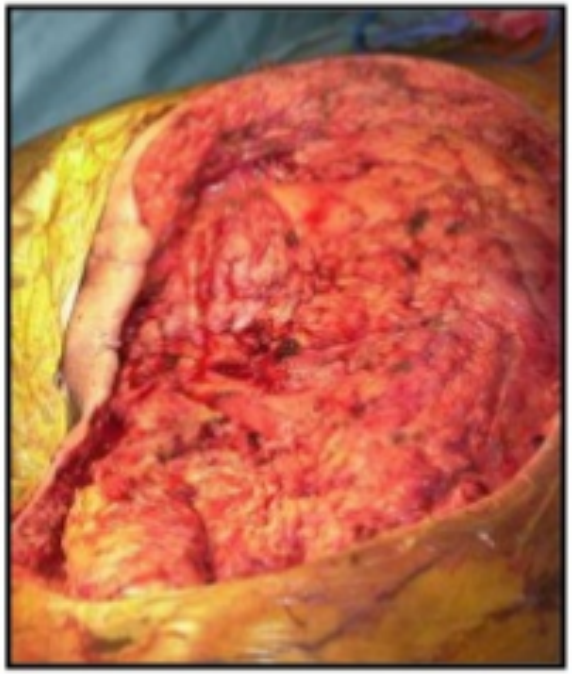
Classification of wound healing

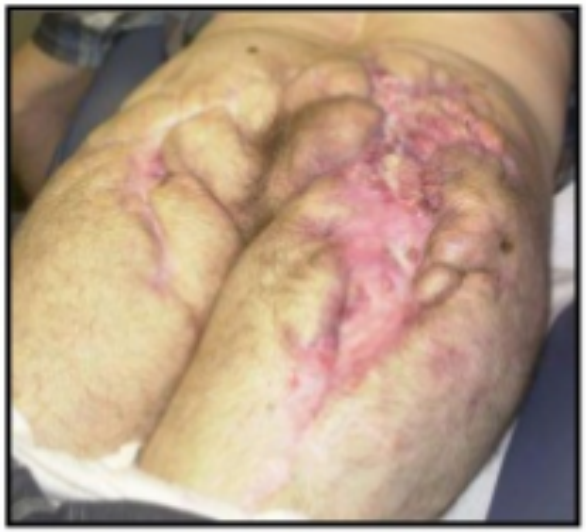


- Secondary Intention
- Occurs when:
  - The edges are separated
  - The gap can not be directly bridged
  - Extensive epithelial loss
  - Severe contamination
  - Significant subepithelial tissue damage
- Healing properties
  - Occurs slowly
  - Granulation; healing from the bottom towards the surface
  - Restoration of the gap by a small amount of scar tissue.
- Scaring
- Wound contracture

Secondary Intention

- For contaminated/dirty wounds
- Wound is intentionally left open
- Healing occurs from the bottom–up
- Granulation tissue containing myofibroblasts forms wound contraction
- Scar formation is extensive



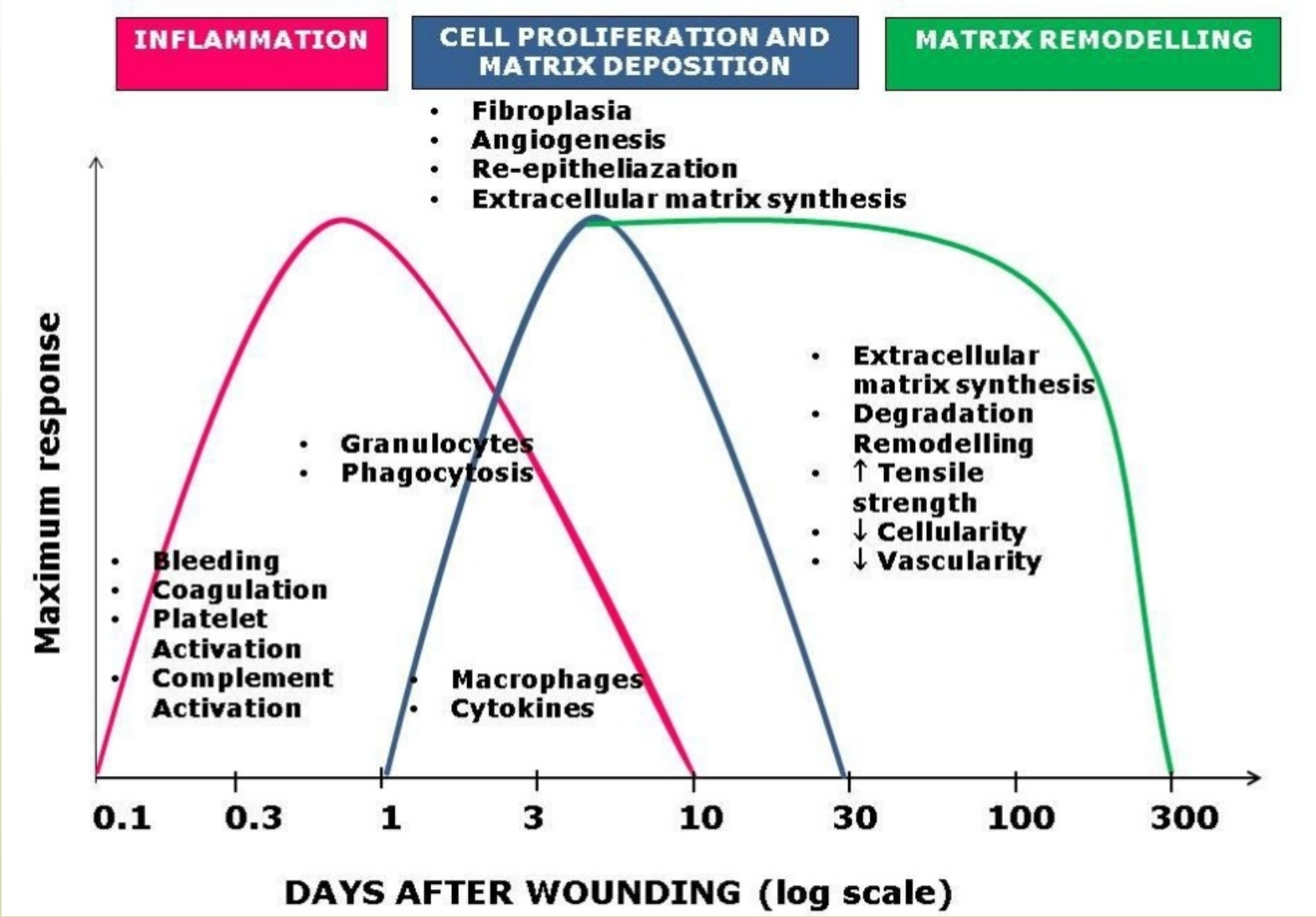


Differences between primary and secondary healing

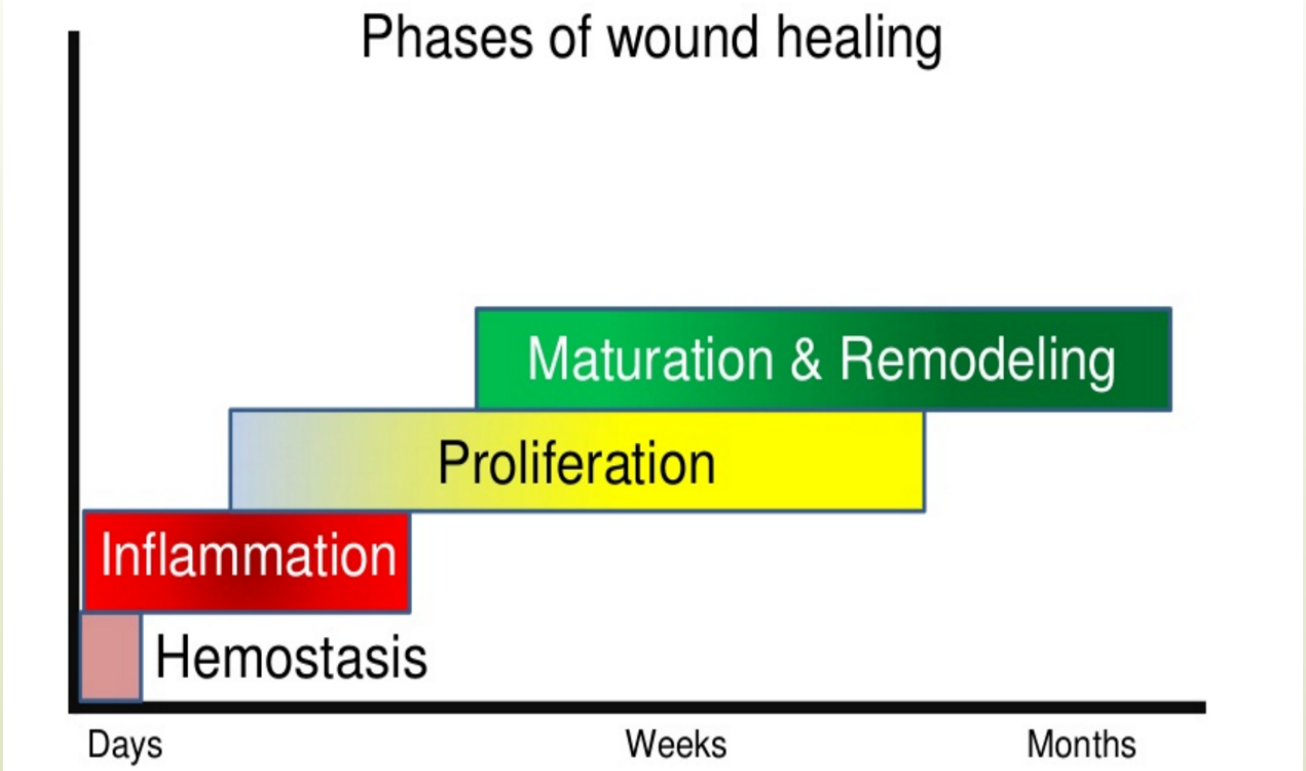
Feature	Primary healing	Secondary healing
Cleanness	Clean	Unclean
Infection	Generally uninfected	May be infected
Margins	Surgically clean	Irregular
Healing	Scanty granulation tissue	Granulation tissue fill the gap
Healing period	Short	long
Healing direction	Direct healing	From the bottom to the edge
Outcome	Neat linear scar	Contracted irregular wound

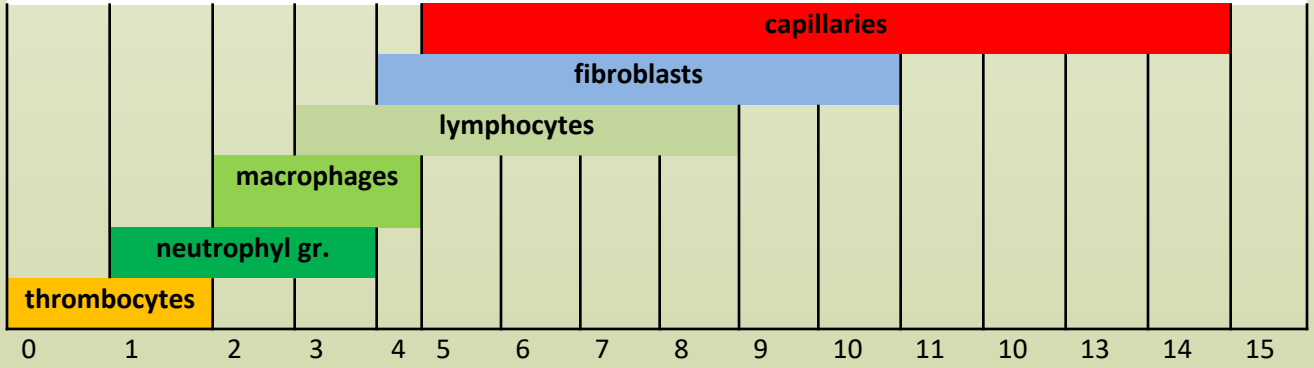
Phases of Healing

- Inflammatory (Reactive)
  - Haemostasis
  - Inflammation
- Proliferative (Regenerative/Reparative)
  - Epithelial migration
  - proliferation
  - Maturation
- Maturational (Remodeling)
  - Contraction
  - scarring
  - Remodeling


INFLAMMATION
CELL PROLIFERATION AND MATRIX DEPOSITION
MATRIX REMODELLING
• Fibroplasia
• Angiogenesis
• Re-epitheliazation
• Extracellular matrix synthesis
Maximum response
• Granulocytes
• Phagocytosis
• Extracellular matrix synthesis
• Degradation Remodelling
• ↑ Tensile strength
• ↓ Cellularity
• ↓ Vascularity
• Bleeding
• Coagulation
• Platelet Activation
• Complement Activation
• Macrophages
• Cytokines
0.1
0.3
1
3
10
30
100
300
DAYS AFTER WOUNDING (log scale)

Normal sequence of wound healing





Growth Factors affecting Wound Healing at Different Stages

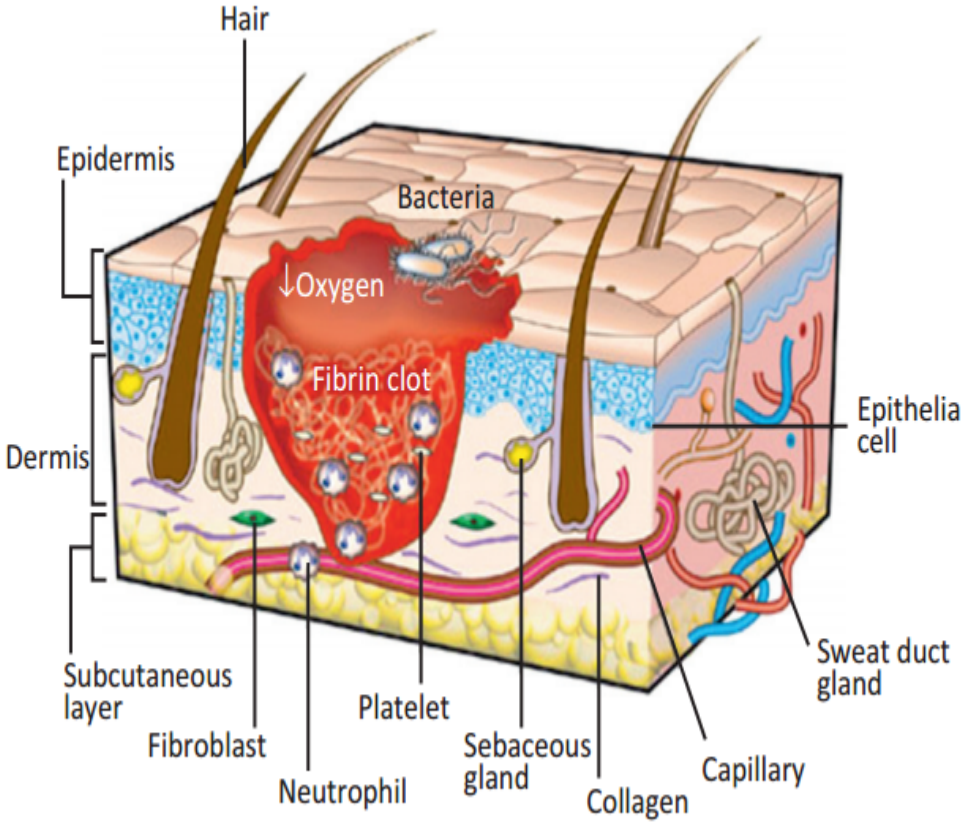
Epithelial Proliferation:	EGF TGFa KGF HGF
Monocyte chemotaxis:	PDGF FGF TGFb
Fibroblast Migration:	PDGF FGF TGFb
Fibroblast Proliferation:	PDGF FGF EGF TNF
Angiogenesis:	VEGF Ang FGF
Collagen Synthesis:	TGFb PDGF
Collagen secretion:	PDGF FGF EGF TNF

TGFb inhibits

Growth Factors in Wound Healing

- Increase size of cells
- Increase number of cells
- Inhibit apoptosis
- Pleiotropic effects i.e initiate cell proliferation, migration, differentiation, contractility, enhance synthesis of specialized proteins eg. Collagen in fibroblasts
- Act in autocrine, paracrine, or endocrine manner

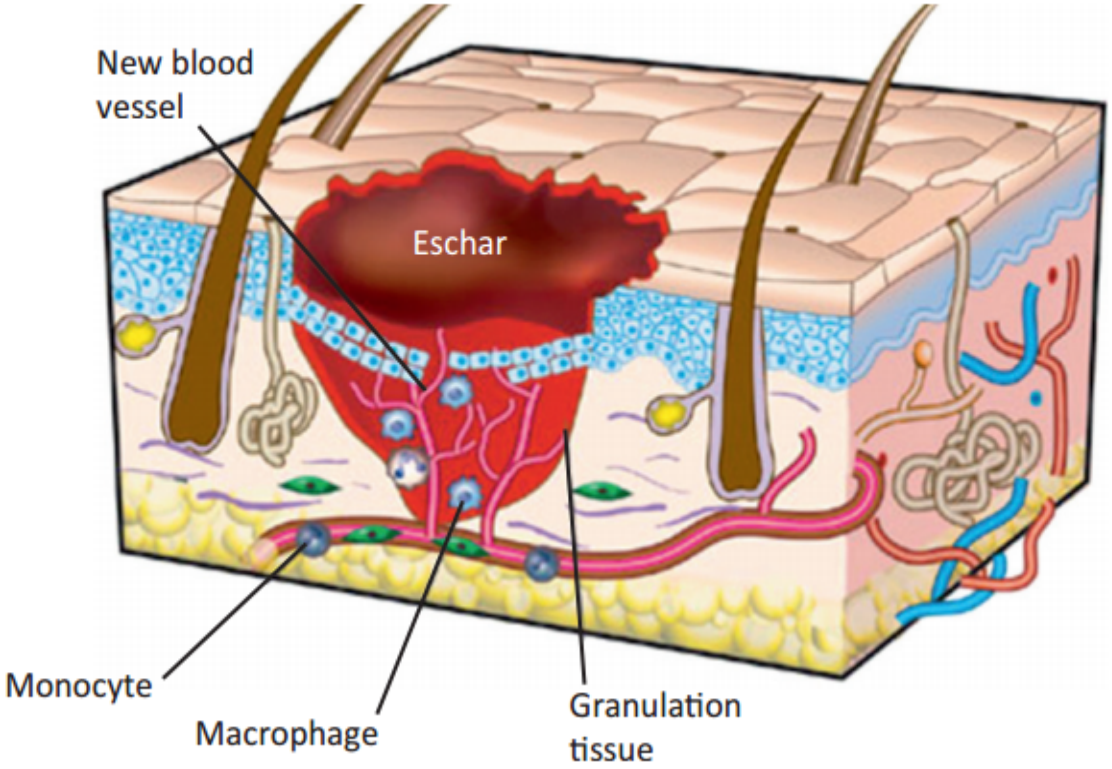
1) The injured area is covered with clot 12-24 hours after injury.



- Coagulation
- Thrombocytes, neutrophils, monocytes, eosinophils...
- Proinflammatory neuropeptides: substance P, neurokinin A
- Histamine, leukotrienes, thrombin
- TGF-β, fibroblast growth factor 2

2) Between 3-7 days after injury, endothelial cells migrate to this region, multiply and new blood vessels form. Fibroblasts migrate to the scar tissue where they are proliferating. The new tissue is called the granulation tissue. Keranocytes multiply at the wound edge and migrate to provisional matrix of damaged dermis.

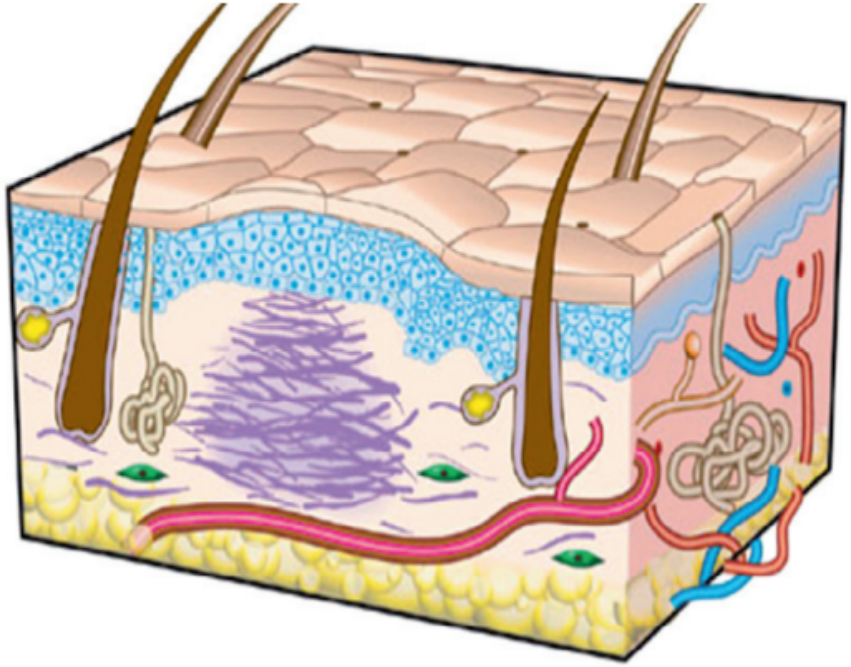
(B) Proliferative phase/reepithelization



- Keratinocyte migration, reepithelization
- FGF-2, -7, -10
- Matrixmetalloproteinase (MMP) 1
- Collagen synthesis, angiogenesis
- Granulation tissue: fibroblasts
- Hypoxia-induced factor (HIF)-1α, VEGF A,

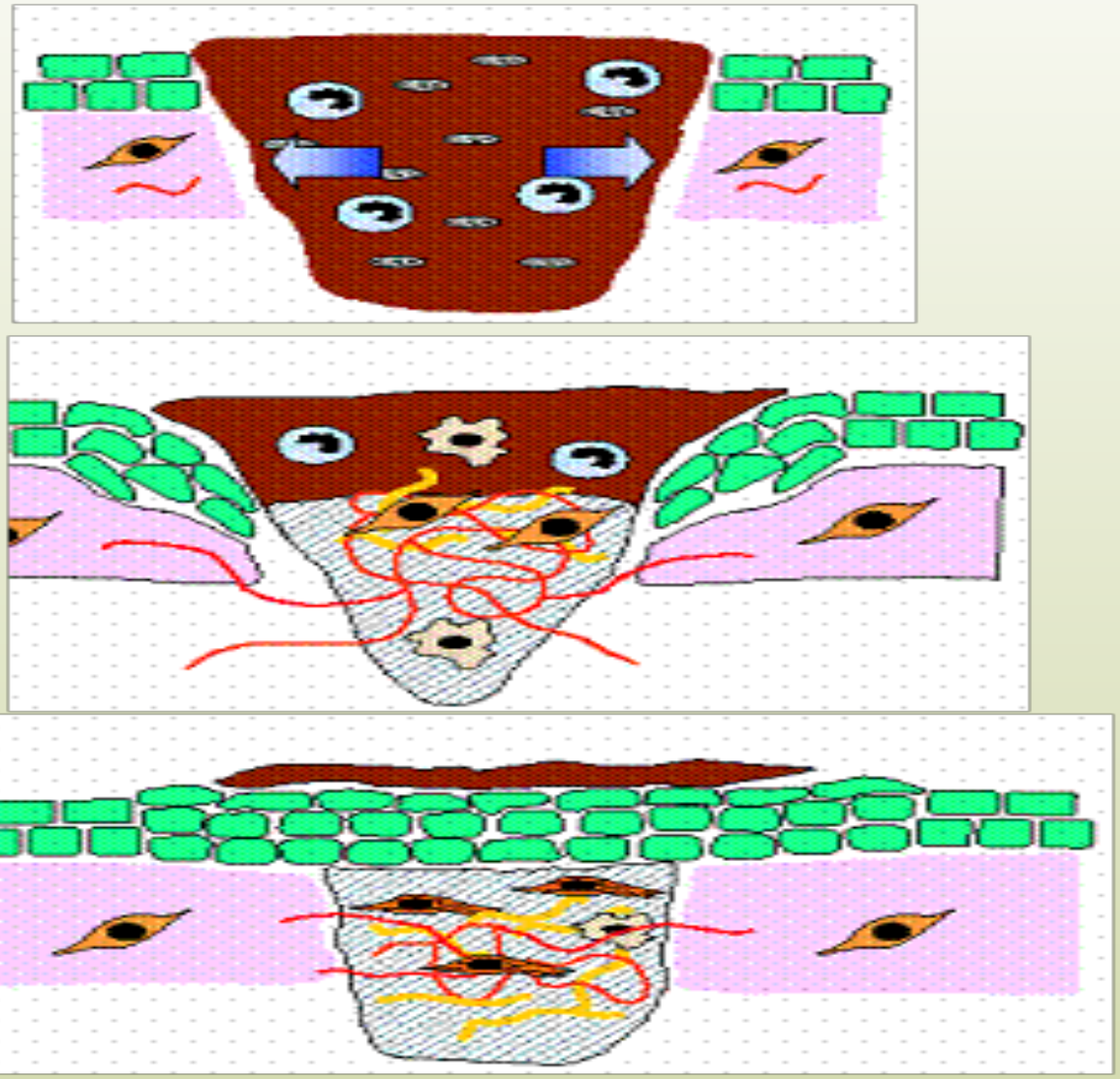
3) One-two weeks after injury, the wound is completely filled with granulation tissue. Fibroblasts become myofibroblasts. This leads to wound contraction and collagen accumulation. The wound is completely covered with new epidermis.

(C) Maturation



- Myofibroblasts → wound contraction
- Platelet-derived growth factor (PDGF), TGF-β
- MMP-2, MMP-7

Wound Healing Phases



Inflammatory phase

- The reaction that occurs immediately after wounding includes a series of defensive events that involves the recognition of a pathogen and the mounting of a reaction against it.
- This reaction involves both coagulation and inflammation

Inflammatory phase

- Coagulation. Apart from an initial period of vasoconstriction lasting for 5-10 minutes, tissue injury causes vasodilation, the disruption of blood vessels and extravasation of blood constituents, including platelets
- The main functions of the exudate are to:
  - Provide cells capable of tissue reconstruction
  - Dilute microbial toxins
  - Remove contaminants present in the wound

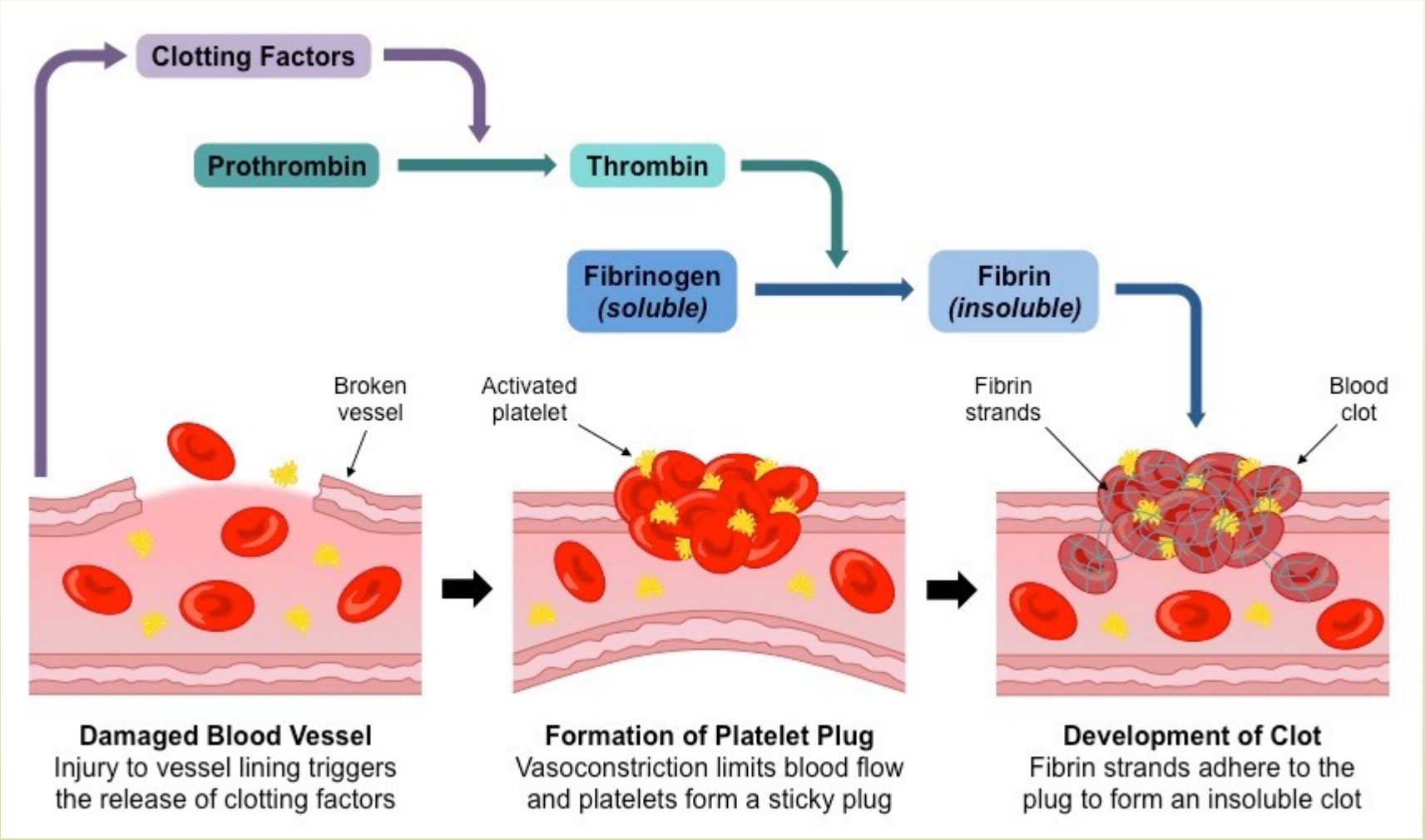
Inflammatory phase

- Coagulation. Shortly after tissue damage, in the first step of wound healing, blood components and tissue factors are released into the wound area.
- The platelets are early modulators of the healing process. They undergo adhesion, aggregation, and activation as a result of their contact with collagen of the damaged vessels, which leads to release of adhesion glycoproteins for platelet aggregation

Blood Cloting

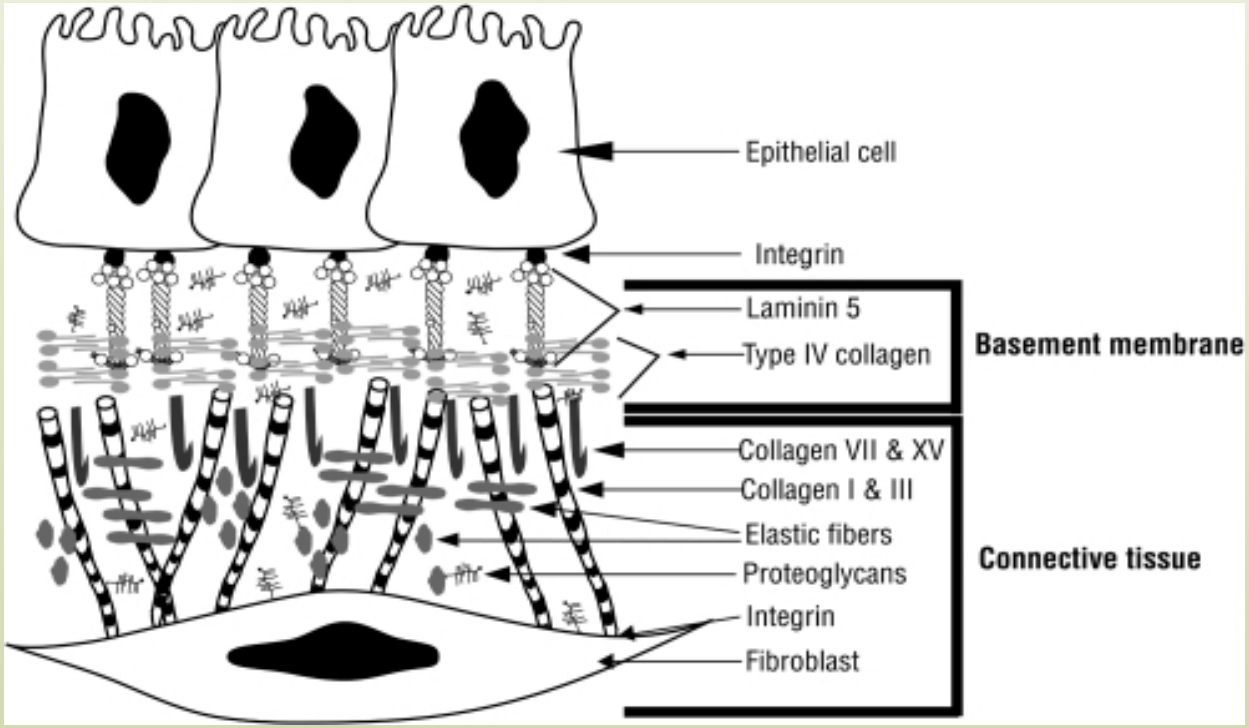
- The key glycoproteins, which are released from α granules of platelets, are fibrinogen, fibronectin, vitronectin, thrombospondin
- The surface of the activated platelets simultaneously becomes the place of prothrombin activation, which leads to creation of active thrombin—the key factor of the coagulation process catalyzing the transformation of fibrinogen into fibrin and as a result of that it forms a blood clot

Blood Cloting



- The blood clot protects the structural integrity of vessels and provides a provisional “scaffolding” which enables formation of a temporary matrix in the wound bed.
- The main component of this temporary, hyaluronan-rich matrix is also plasma **fibronectin**, which is accumulated in the wound during the first 24 hours after the injury
- The polymerized fibronectin shows highly adhesive properties entering the interaction with numerous cells by integrin receptors and stimulates the migration and adhesion of fibroblasts, keratinocytes, and endothelial cells.

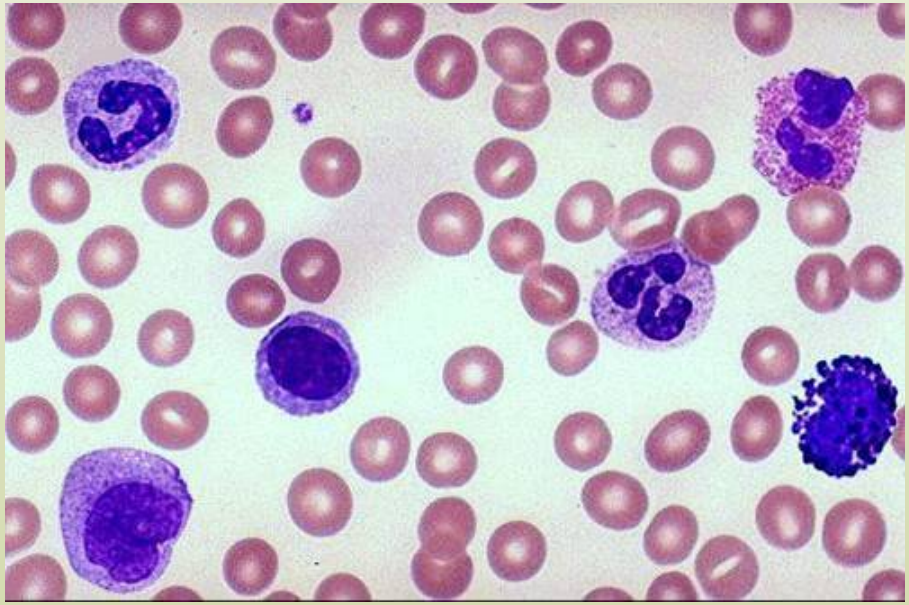
- Cell-ECM interactions are equally important in closing epithelial wounds. 24 hours after injury, accumulation of fibronectin occurs, followed by **collagen type IV** and **laminin** deposition to form basement membrane. In this way, matrix adhesion and migration are closely related to wound closure of the matrix deposition.



- Fibroblasts originate from the connective tissue and travel along the fibrin filaments to the wound area. During cutaneous wound closure, keratinocytes migrate primarily through a transient matrix of fibronectin, vitronectin, tenascin and collagen type III.

Inflammation phase

- Neutrophils
- Macrophages
- Lymphocytes



Neutrophils

- Neutrophils are the first inflammatory cells that arrive the wound bed.
- It is visible in the wound after 6 hours
- Neutrophil sterilizes the bacteria and necrotic tissues for three days by phagocytosis.

Macrophages

- Macrophages participate in phagocytosis and process of killing bacteria or removing debris, by secreting matrix metalloproteinases, for example, collagenase, or elastase.
- They are the source of TGF-β also secreting PDGF, TGF-α, bFGF, HB-EGF, IL-1, IL-6, and TGF-α.
- stimulating the proliferation of fibroblasts and collagen biosynthesis,
- modulate the epithelialization, collagen accumulation, and angiogenesis

Lymphocytes

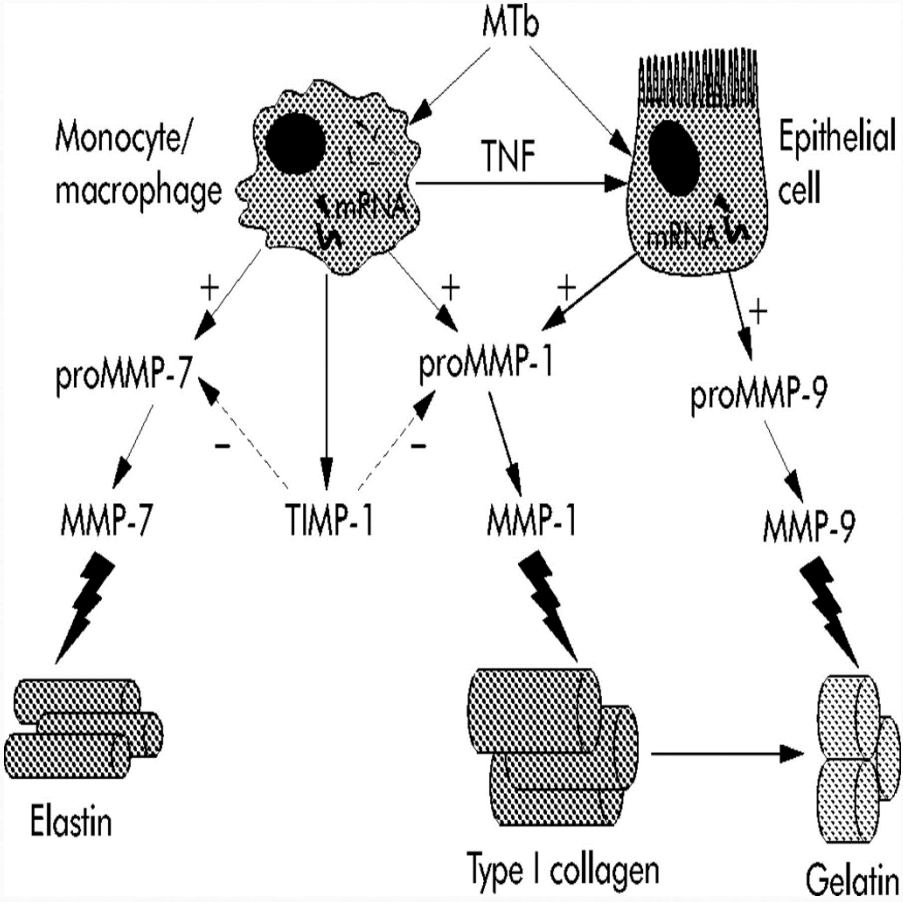
- In the late inflammatory phase, lymphocytes also infiltrate the wound environment influencing fibroblast proliferation and collagen biosynthesis

MMPs (Matrix metalloproteinases)

- Part of a larger family of Metalloproteinases that play an important role in wound healing.
- They are produced by inflammatory cells (Neutrophils & macrophages) and wound cells (epithelial, fibroblasts and vascular endothelial cells).
- When first synthesised, MMPs are latent. They are activated by other proteases.
- 23 MMPs have been identified. MMP – 1, 2, 8 & 9 are related to wound healing.

Matrix Metalloproteinases (MMPs)

- Essential for the migration of cells through the ECM
- They remove collagen and other ECM components that were denatured during injury
- Important because collagen molecules must interact with each other to form a fibril (Fine fibre)
- Partially degraded matrix will not bind resulting in disorganised, weak ECM
- Degraded collagen must be removed by the controlled action of MMPs
- Hole in the wall image…



Proliferative Phase

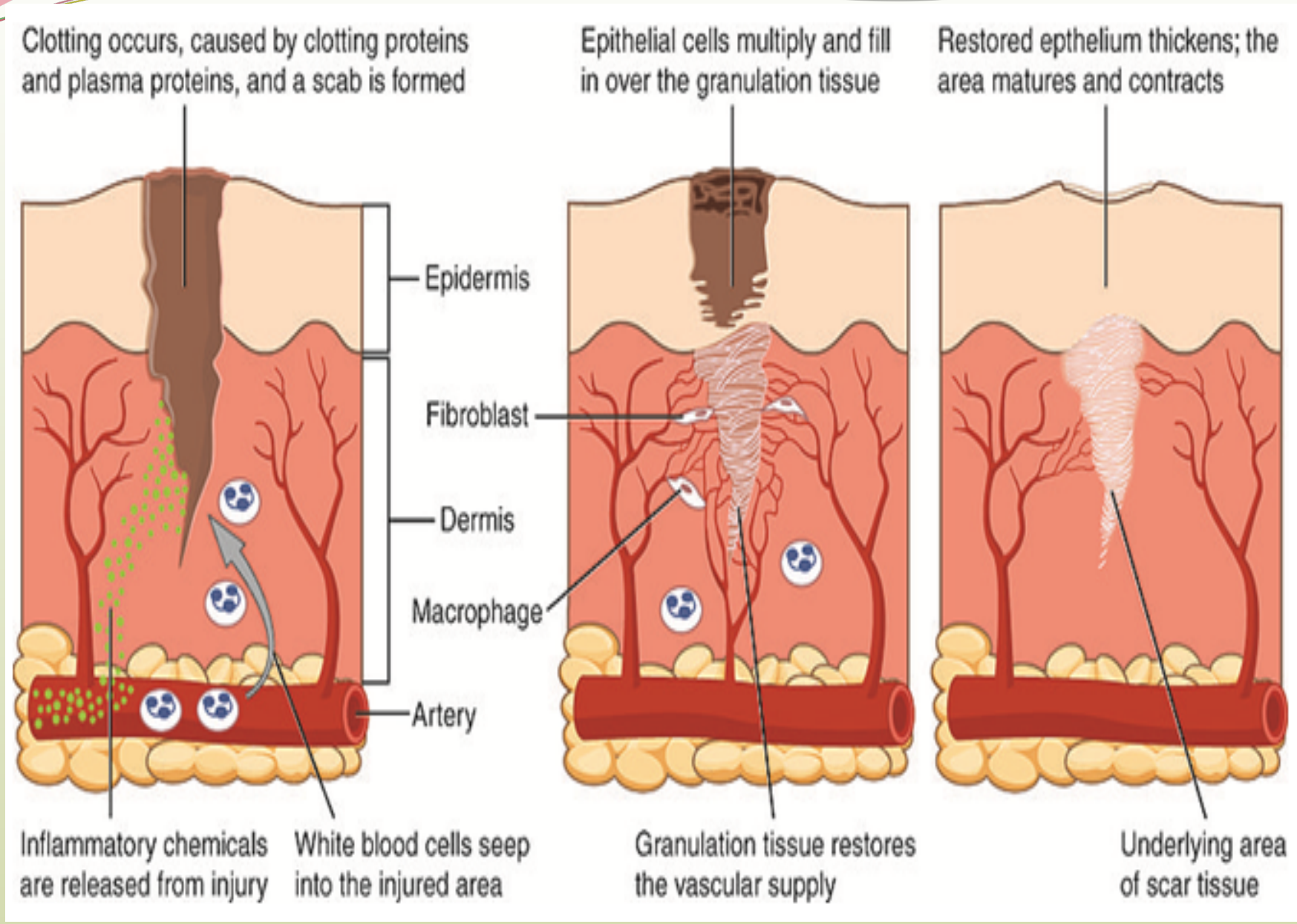
- As the healing progresses, fibronectin produced by macrophages and fibroblasts contributes to the formation of granulation tissue in the wound bed.
- These matrix molecules act as substrates in the migration of endothelial cells
- Endothelial cells form vessels in the wound bed.

Granulation Tissue

- Fibroblasts,
- Myofibroblasts,
- Monocytes / macrophages,
- Lymphocytes, Microvessel
- Endothelial cells and
- ECM molecules (embryonic fibronectin, hyaluronic acid, type III collagen, and small amounts of type I collagen)

These ECM molecules lead to proliferation of fibroblast, epithelium and endothelial cells by providing signals to the cells with growth factors secreted by platelets and other cells in the granulation tissue. This stage is called as fibroplasia

The main function of fibroblasts is **collagen synthesis**. This synthesis starts on the second day of the injury and shows the highest activity on 5-7 days.


Clotting occurs, caused by clotting proteins and plasma proteins, and a scab is formed
Epithelial cells multiply and fill in over the granulation tissue
Restored epthelium thickens; the area matures and contracts
Epidermis
Fibroblast
Dermis
Macrophage
Artery
Inflammatory chemicals are released from injury
White blood cells seep into the injured area
Granulation tissue restores the vascular supply
Underlying area of scar tissue

- PDGF and TGF-β, which is also secreted by blood platelets and macrophages, regulates the accumulation of ECM components

- The matrix of the early granulation tissue (up to the third day after the injury) contains great amount of hyaluronic acid and fibronectin.

- The hyaluronic acid molecules, which are characterized by an ability to swelling, create a woven structure which enables the coming cells to penetrate the wound area

- Fibronectin also facilitates the fibrogenesis of collagen.
- Starting with the third day after the injury, the concentration of hyaluronic acid within the wound area quickly decreases, while collagen takes the place of this glycosaminoglycan.
- The collagen content in the granulation tissue increases up to the third week.
- That is accompanied by a gradual decrease of the fibroblast when they disappear in the process of apoptosis

Epithelization

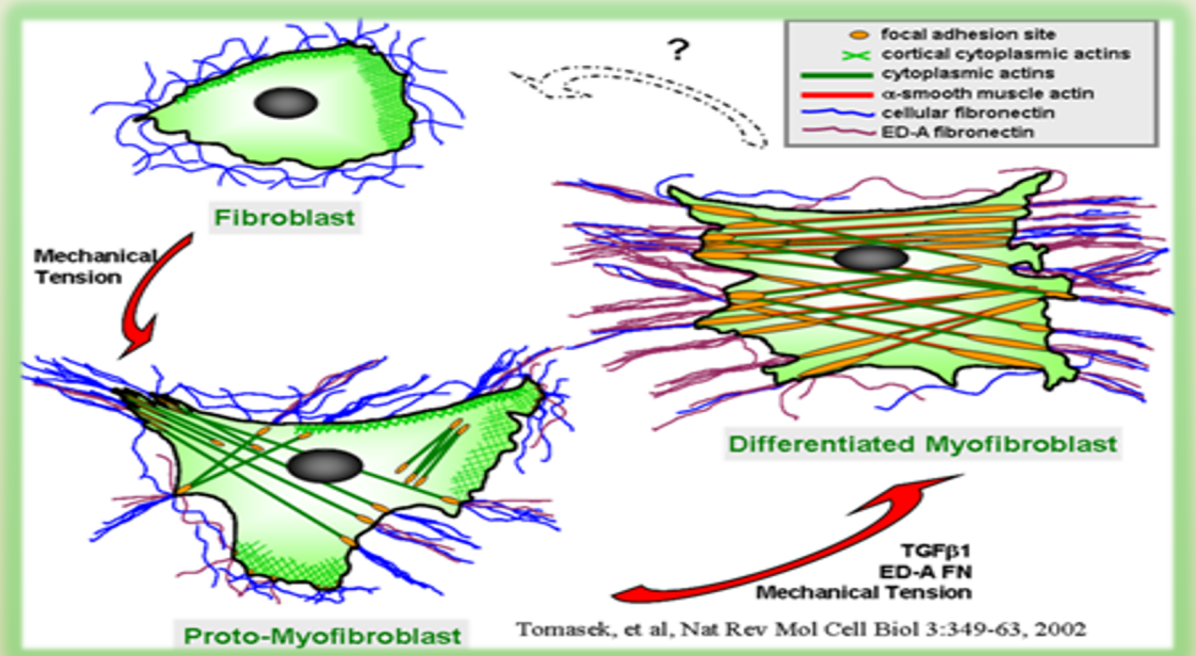
- Epithelization is the division of the lower layers of the skin tissue and the covering of the granulation tissue. Epithelization begins 24 hours after injury.
- Extracellular matrix, growth factors, and changes in the electrical area generated by the wound stimulate the migration of epithelial cells.
- After covering the wound surface, the epidermis begins keratinization. Keratinocytes and fibroblasts secrete laminin and type IV collagen to form the basal membrane.

Angiogenesis

- Angiogenesis is a key phase of the healing process. In the course of this process, endothelial cells migrate to the temporary matrix of the wound
- Without angiogenesis, there will be no invasion of macrophages and fibroblasts into the wound bed because there will be no oxygen and nutrients. Therefore, angiogenesis continues until the end of wound healing.

Remodeling

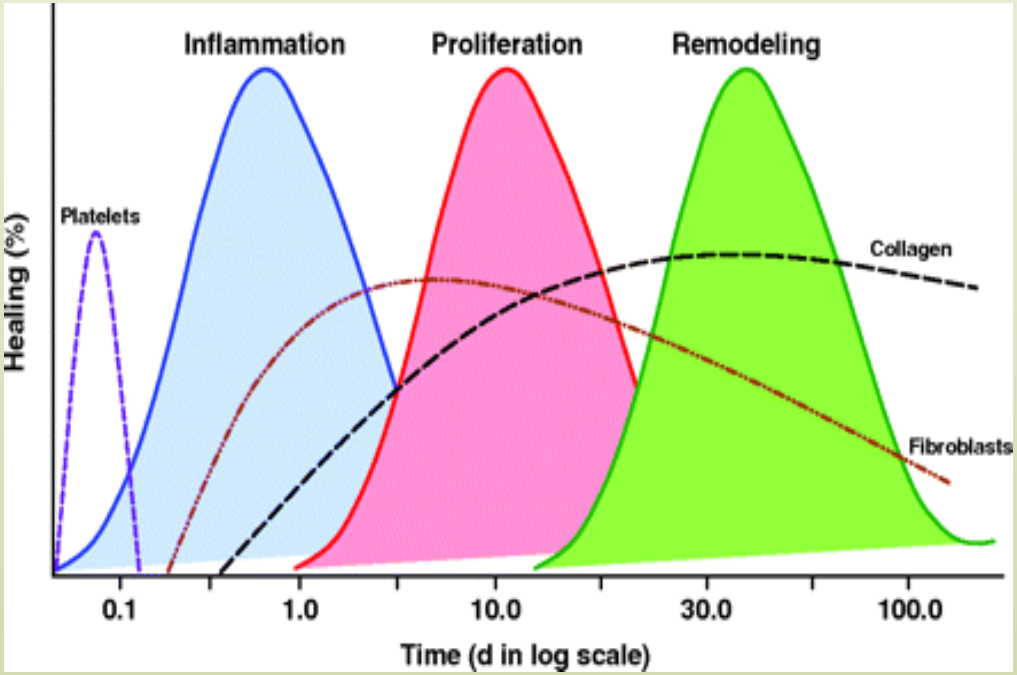
- ✓ Remodeling is the last phase of the healing process. In its course, the wound surface is contracted. The key phenomenon of wound contracture is phenotypic differentiation of the preexisting fibroblasts into myofibroblasts. The latter ones contain fibrils of alpha smooth muscle actin (α-sma) microfilaments, which give the cells the property of contracting

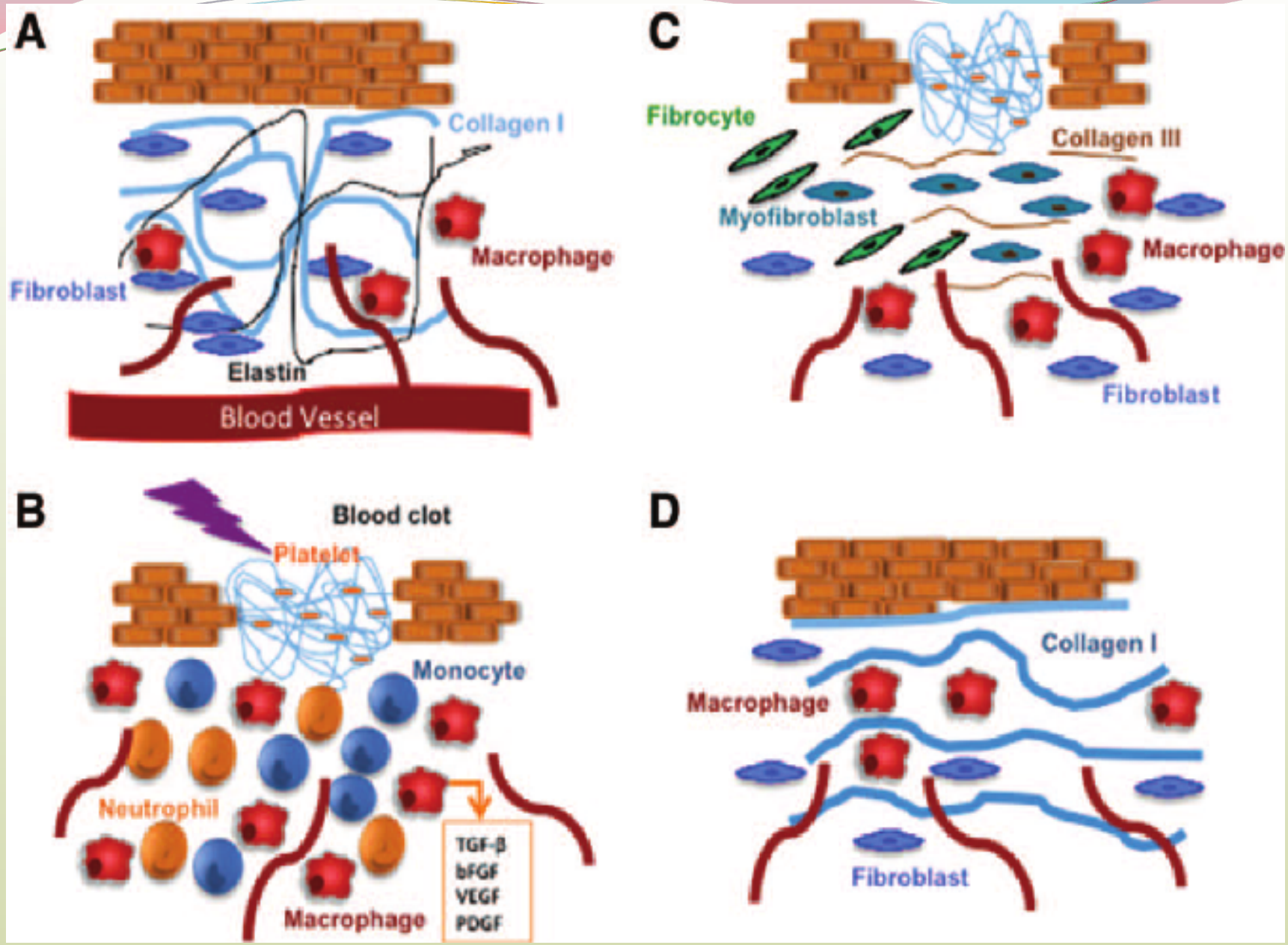


- ✓ During this phase of the healing process, the granulation tissue "matures" to the form of a scar, which is accompanied by the increase of mechanic strength of the formed tissue.
- ✓ The mutual proportion of collagen types changes (type I collagen content increases in favor of collagen type III), the total collagen content increases, its spatial organization becomes arranged, and the number of covalent cross-links increases, which leads to increased tensile strength of the tissue.

Apoptosis

- Apoptosis plays an important role in the conversion of granulation tissue to scar tissue.
- When the wound heals, the number of fibroblasts, myofibroblasts, endothelial cells, pericytes decreases considerably, matrix molecules, especially interstitial collagen accumulate and scarring occurs.
- Cell death by apoptosis during remodeling leads to the elimination of many different types of cells at the same time without causing tissue damage.




A
Collagen I
Macrophage
Fibroblast
Elastin
Blood Vessel
B
Blood clot
Platelet
Monocyte
Neutrophil
Macrophage
TGF-β
bFGF
VEGF
PDGF
C
Fibrocyte
Collagen III
Myofibroblast
Macrophage
Fibroblast
D
Collagen I
Macrophage
Fibroblast

Wound Healing Phases

Inflammatory

1) Immediate to 2-5 days
2) Bleeding stops (haemostasis)
   - i Constriction of the blood supply
   - ii Platelets start to clot
   - iii Formation of a scab
3) Inflammation
   - i Opening of the blood supply
   - ii Cleansing of the wound

Proliferative

1) 5 days to 3 weeks
2) Granulation
   - i New collagen tissue is laid down
   - ii New capillaries fills in defect
3) Contraction
   - i Wound edges pull together
4) Epithelialization
   - i Cells cross over the moist surface
   - Ii Cell travel about 3 cm from point of origin

Maturation

1) Collagen forms which increases tensile strength to wounds
2) Scar tissue is only 80 percent as strong as original tissue
3) 3 weeks to 2 years

SIGNAL TRANSDUCTION DURING CELL-ECM INTERACTIONS

In studies up to now, it has been shown that ECM molecules interact with cell surface receptors and transmit signals directly or indirectly via a secondary messenger.

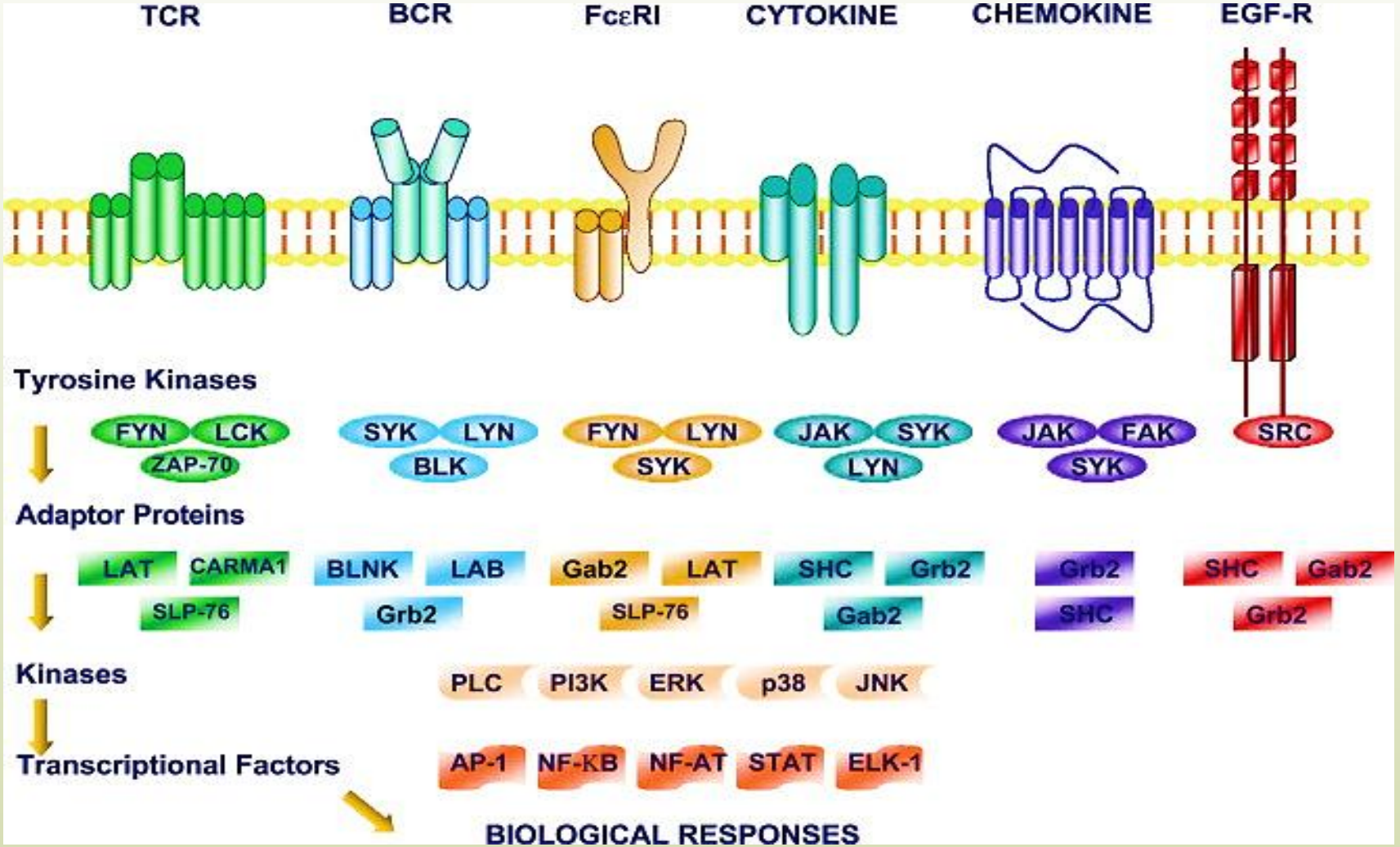
Secondary messengers illuminate the sequence of events leading to coordinated expression of various genes that cause cell adhesion, migration, proliferation, differentiation and death.

Signal transmission is a network that occurs between pathways associated with growth factor receptors, integrins and G protein-coupled receptors (GPCRs).

- *Receptor tyrosine kinases (RTK) phosphorylate tyrosine residues from substrate proteins.*

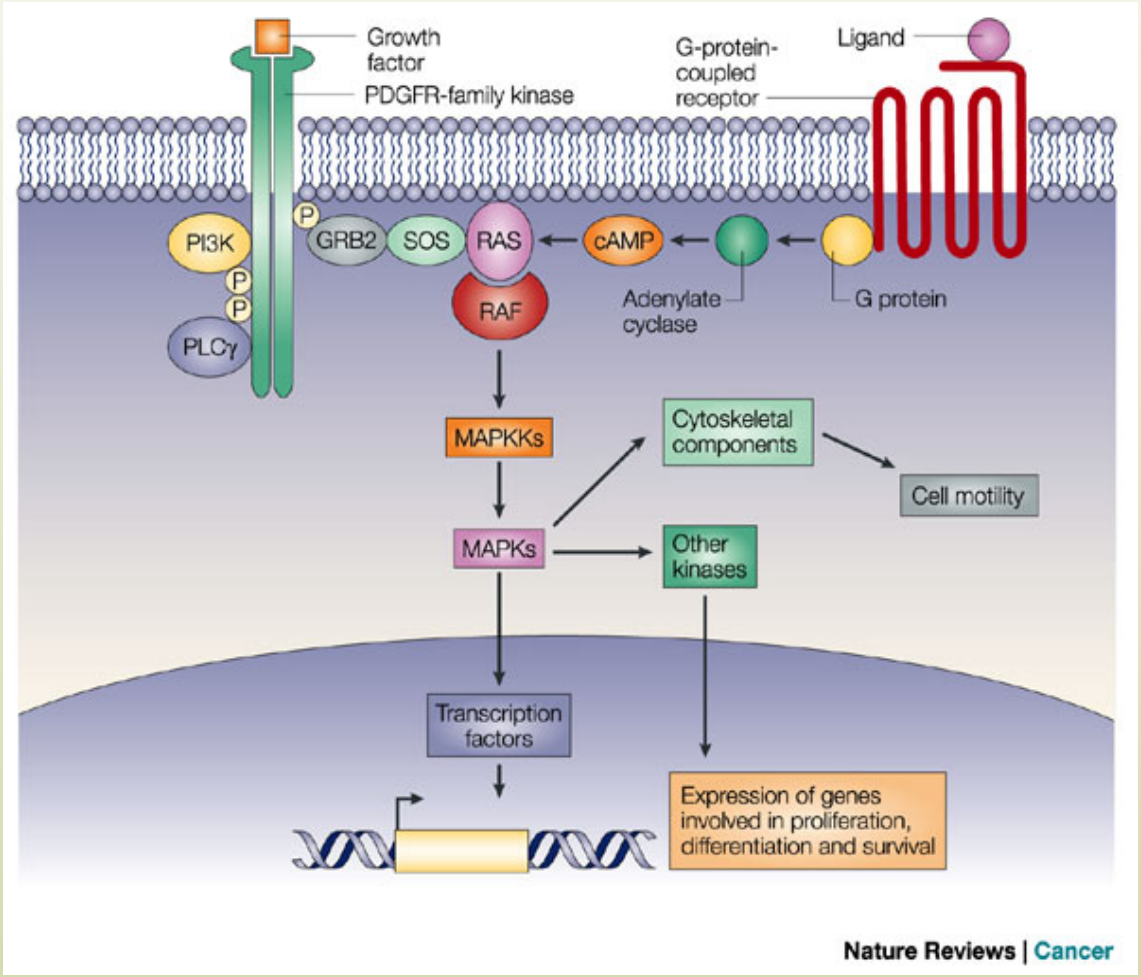
- *EGF (Epidermal growth factor), NGF (Neuronal growth factor), PDGF (Platelet derived growth factor), insulin and some growth factor receptors are tyrosine kinases.*
- There are two classes of tyrosine kinases: 1) receptor tyrosine kinases; 2) non-receptor tyrosine kinases. The first group are transmembrane proteins with a ligand binding extracellular domain and a catalytic intracellular kinase domain. The second group is found in the cytosol, the nucleus and the inner surface of the plasma membrane.

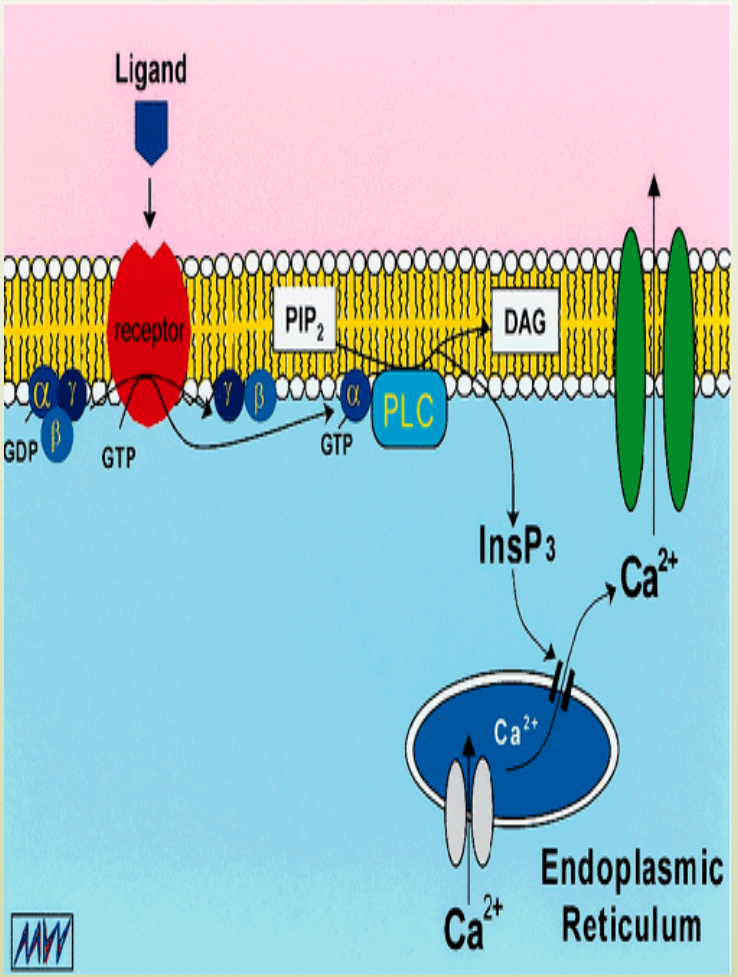
- The RTK binds to the signaling protein containing the SH2 (src homology-2) region via the phosphorylated tyrosines on them.
- This interaction is like a well-customized key lock compatibility. For example, the SH2 region of Src recognizes phosphotyrosine - glutamic acid - glutamic acid - isoleucine motifs.
- Other proteins containing SH2 include the Grb2 adapter protein, phospholipase C (PLC) and phosphoinositide-3-kinase (PI3K). The signal is transmitted to the proteins by the Grb2 and Sos adapter proteins and the non-receptor tyrosine kinases (Src) that come together by the receptor.


TCR
BCR
FcεRI
CYTOKINE
CHEMOKINE
EGF-R
Tyrosine Kinases
FYN
LCK
ZAP-70
SYK
LYN
BLK
FYN
LYN
SYK
JAK
SYK
LYN
JAK
FAK
SYK
SRC
Adaptor Proteins
LAT
CARMA1
SLP-76
BLNK
LAB
Grb2
Gab2
LAT
SLP-76
SHC
Grb2
Gab2
Grb2
SHC
SHC
Gab2
Grb2
Kinases
PLC
PI3K
ERK
p38
JNK
Transcriptional Factors
AP-1
NF-KB
NF-AT
STAT
ELK-1
BIOLOGICAL RESPONSES

- The active secondary messenger in the intracellular signaling pathway is from a phospholipid, PIP2 (phosphatidylinositol 4,5 biphosphate). Hydrolysis of PIP2 with the enzyme phospholipase C (PLC) results in the formation of two secondary reporters, diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP3). These two messengers activate two different signaling pathways: protein kinase C (PKC) and calcium (Ca ++) release.

- PKC activates other intracellular targets, such as the MAP Kinase pathway, and causes phosphorylation in transcription factors that affect gene expression and cell proliferation.

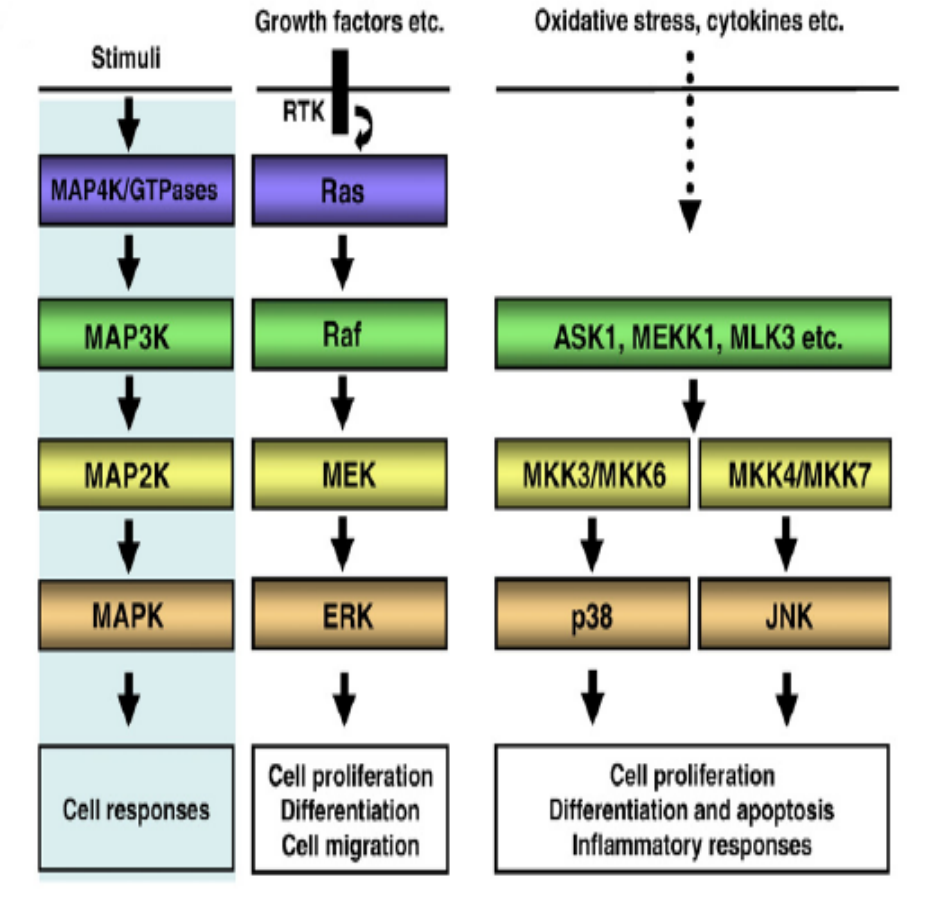




- MAP kinases were serine and threonine protein kinases that were activated by growth factors and other signaling molecules and translocated to the nucleus. Upon activation by mitogenic signaling pathways, MAPKs go to the nucleus and induce transcription factors to bind to DNA.

- There are at least three subgroups of the MAPK family: 1) ERKs (extracellular signal-regulated kinase), 2) P38 and 3) Jnk (c-Jun NH2-terminal kinase).

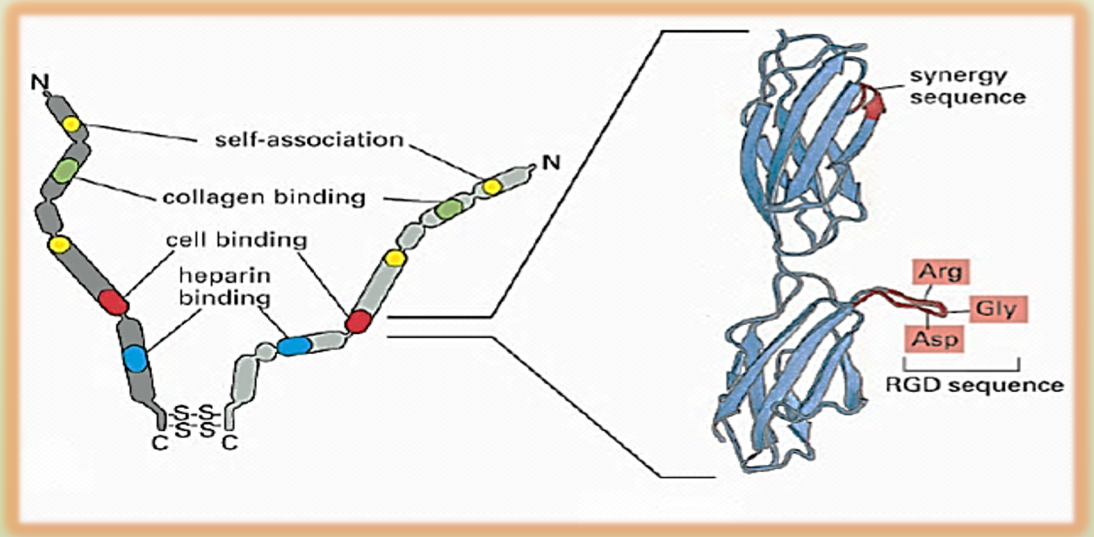
- For ERK activation, two protein kinases are involved: Raf, followed by MEK (MAP kinase or ERK kinase).

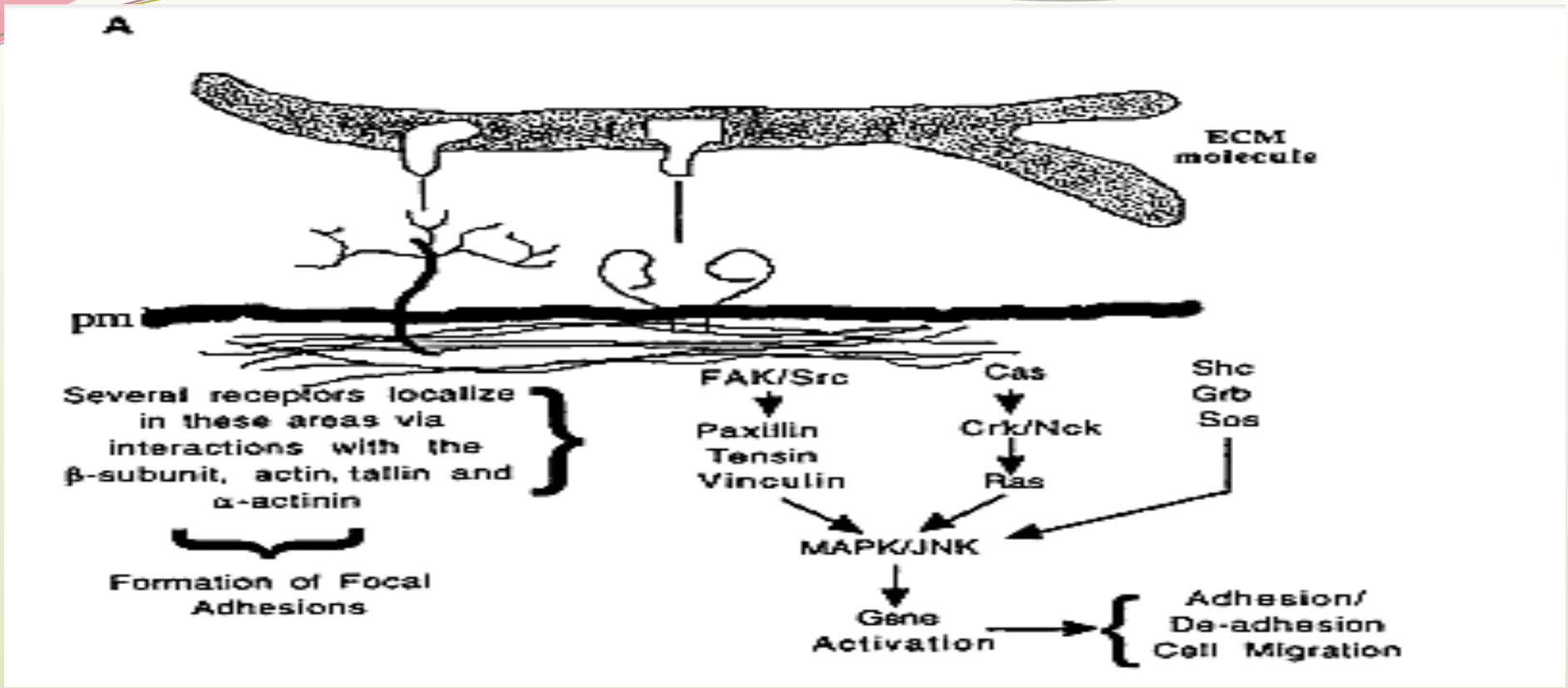


Cell receptor-ECM interactions leading to cellular events are examined in three categories:

1) Type I Interactions contain integrins and proteoglycan receptors and participate in cell adhesion / deadhession processes during migration.

For example, cell migration is promoted when fibronectin is bound to integrins along the cell-binding domain and to the proteoglycan receptors along the heparin binding site.

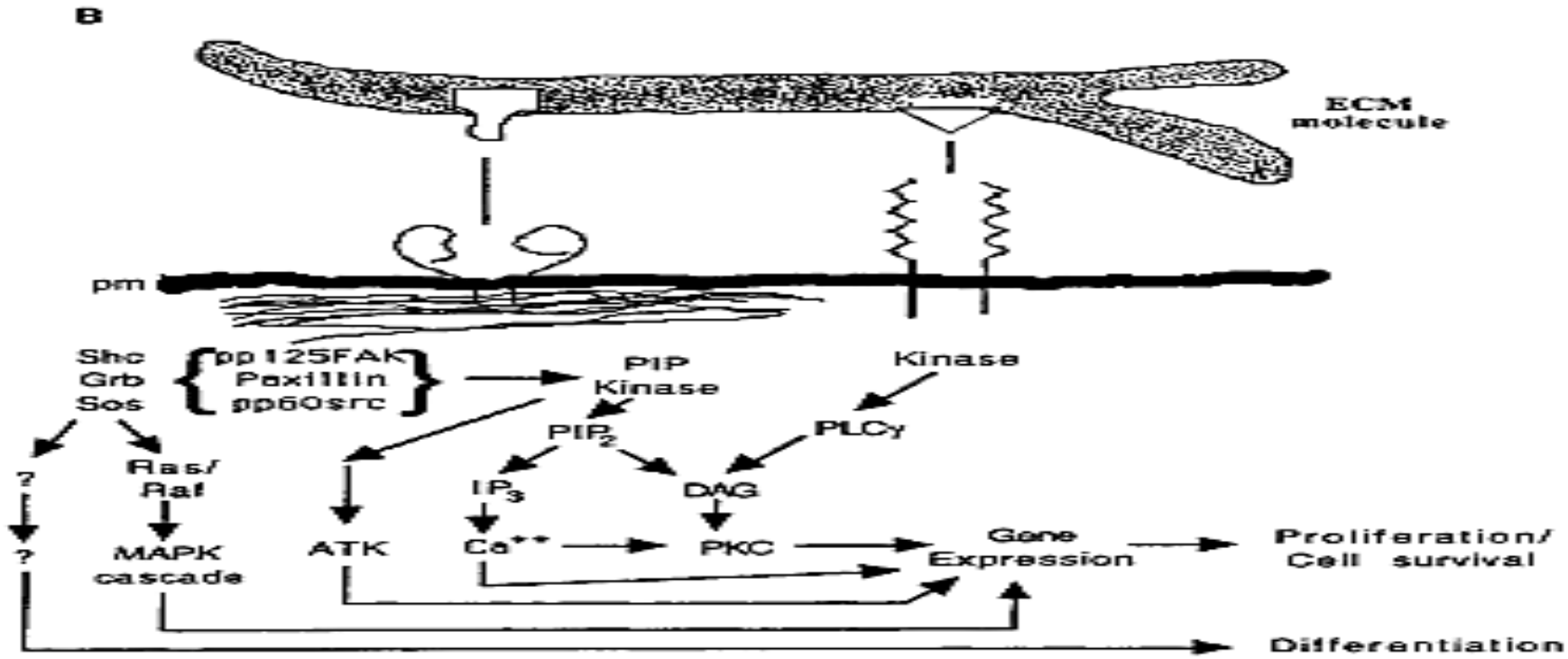




(A) In the focal adhesion, the proteoglycan (tree-like structure) and integrin (heterodimer) receptors on the plasma membrane (pm) bind to different epitopes in the same ECM molecule. This binding leads to the reorganization of the cytoskeletal. Many protein phosphorylation (eg, pp 125 FAK, Src, PKC) discloses gene activation pathways that are important for migration, adhesion / deceased.

2) Type II interactions include processes that affect survival and proliferation, as well as differentiation and continuity of differentiated phenotypes.

In these processes, the extracellular matrix interacts with its receptors and cooperates with growth factors and cytokine receptors. These cooperative effects occur, for example, during anchoring-dependent cell growth.



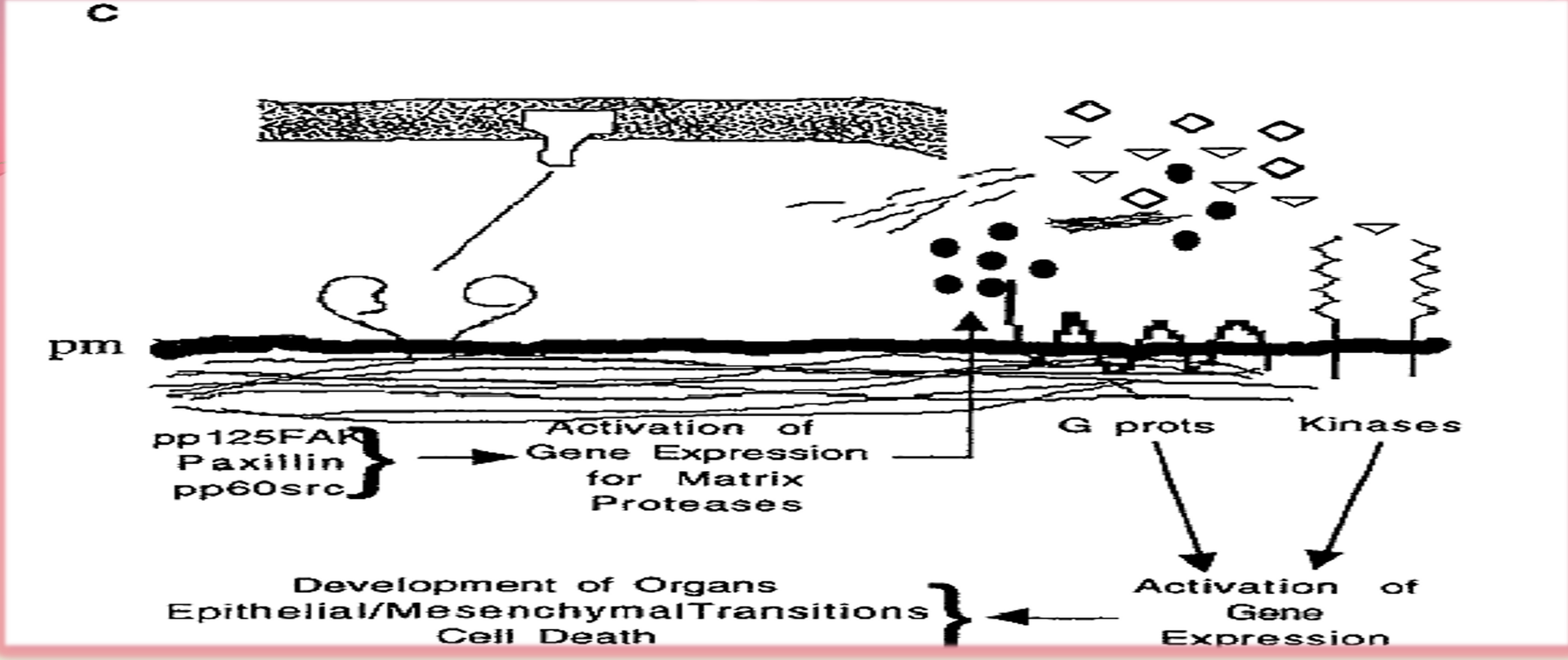
(B) The ECM molecule binds to integrin receptors via specific ligands to activate the cytoskeletal elements. On the other hand, growth factors linked to matrix molecules are bound to receptors with kinase activity.

Phospholipase C becomes active. Activated phospholipase C (PLC) catalyzes the translation of phosphatidyl inositol biphosphate (PIP2) to two secondary messengers known as, inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 binds to receptors on the smooth endoplasmic reticulum, inducing the release of intracellular calcium. These Ca ions either directly lead to gene expression or indirectly work with protein kinase C (PKC) leading to gene expression. In this case, gene activation is important in cell proliferation, differentiation and protection of differentiated phenotypes.

3) Tip III interactions often involve processes leading to cell death and mesenchymal transition from epithelia.

Signal transduction pathways lead to apoptosis, which is designed for endothelial cells and leukocytes, and appears to primarily involve tyrosine kinase activity.

Remodeling of the matrix during the transition from epithelial cells to mesenchymal cells is an important component of the interaction. The enzymatic degradation of the ECM contributes to the release of the soluble components and components of the ECM, which contain specific sequences that affect cell behavior.

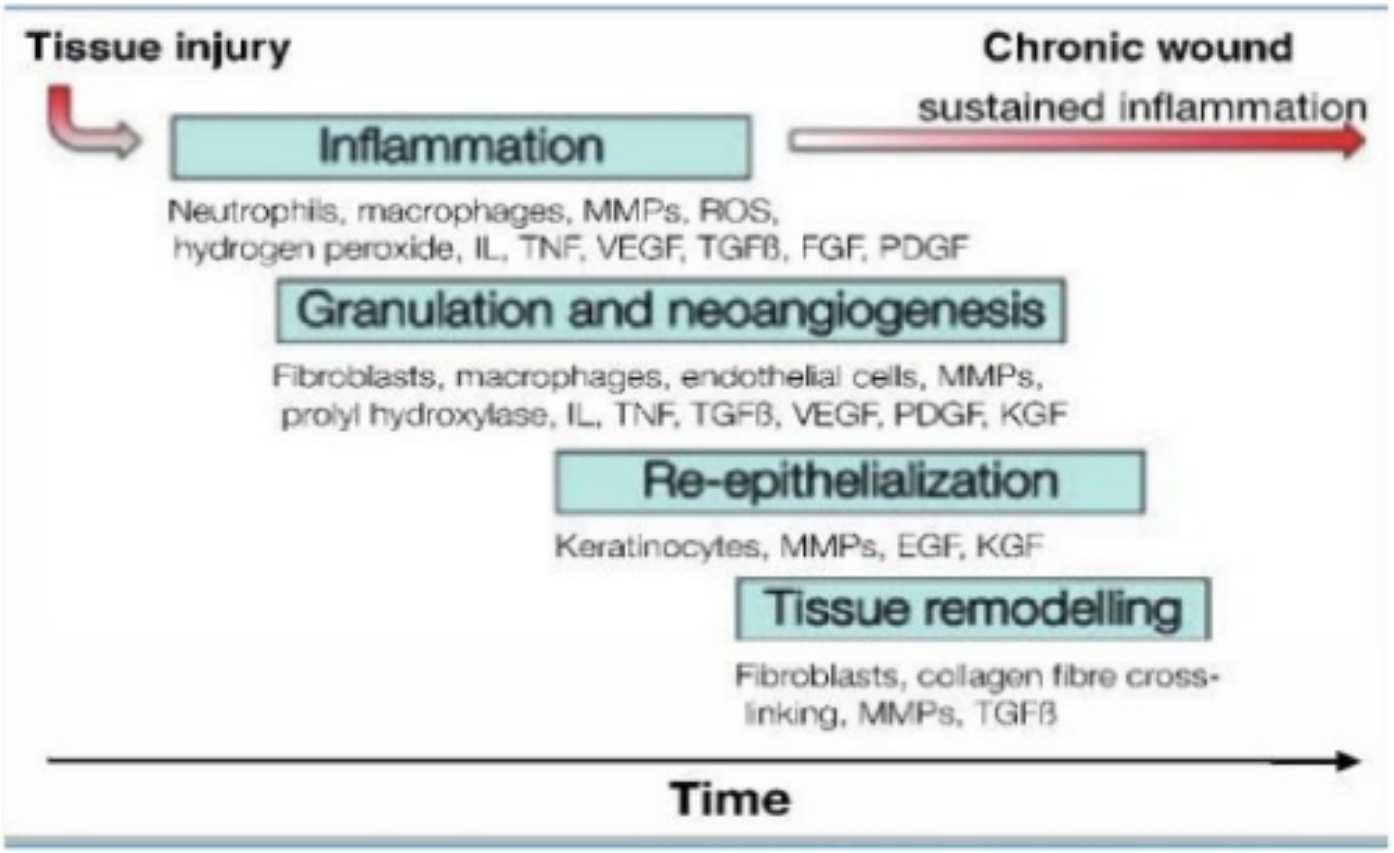


(C) Integrin receptors bind to ECM molecules that contain specific domains. This binding leads to the activation of matrix protease genes. The resulting products (black ellipse shaped) degrade the matrix and release the peptide (line shaped), growth factors (triangle and tile). These peptides and growth factors then interact with specific cell surface receptors to activate G proteins and kinases. This activation also leads to the expression of genes important in cell death and morphology.

Suitability of ECM for Tissue Engineering

1) *ECM can prevent inflammation and tissue rejection*
2) *ECM can provide suitable ground for cellular differantiation and survival*
3) *ECM can provide appropriate environment for preventing tissues from outer stimulants*

The healing progression of chronic wounds usually becomes arrested in this inflammatory stage



RISK FACTORS FOR WOUND INFECTION

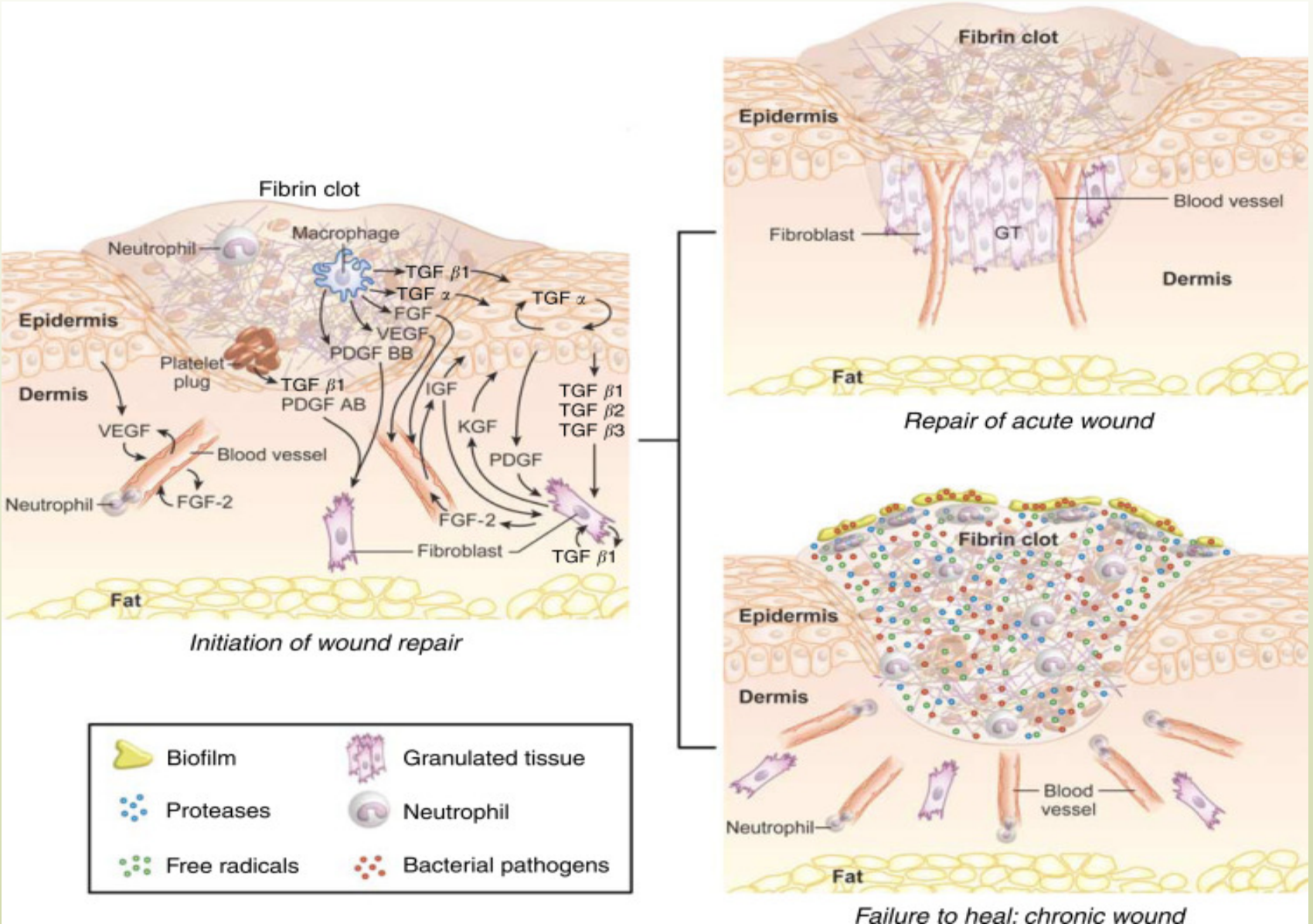
SYSTEMIC

- Vascular disease
- Edema
- Malnutrition
- Diabetes
- Alcoholism
- Prior surgery or radiation
- Drugs i.e. corticosteroids
- Immune deficits

LOCAL

- Large wound area
- Increased wound depth
- Degree of chronicity
- Anatomic location
- Foreign bodies
- Necrotic tissue
- Mechanism of injury
- Degree of contamination
- Reduced perfusion

Failure of Chronic Wound Healing



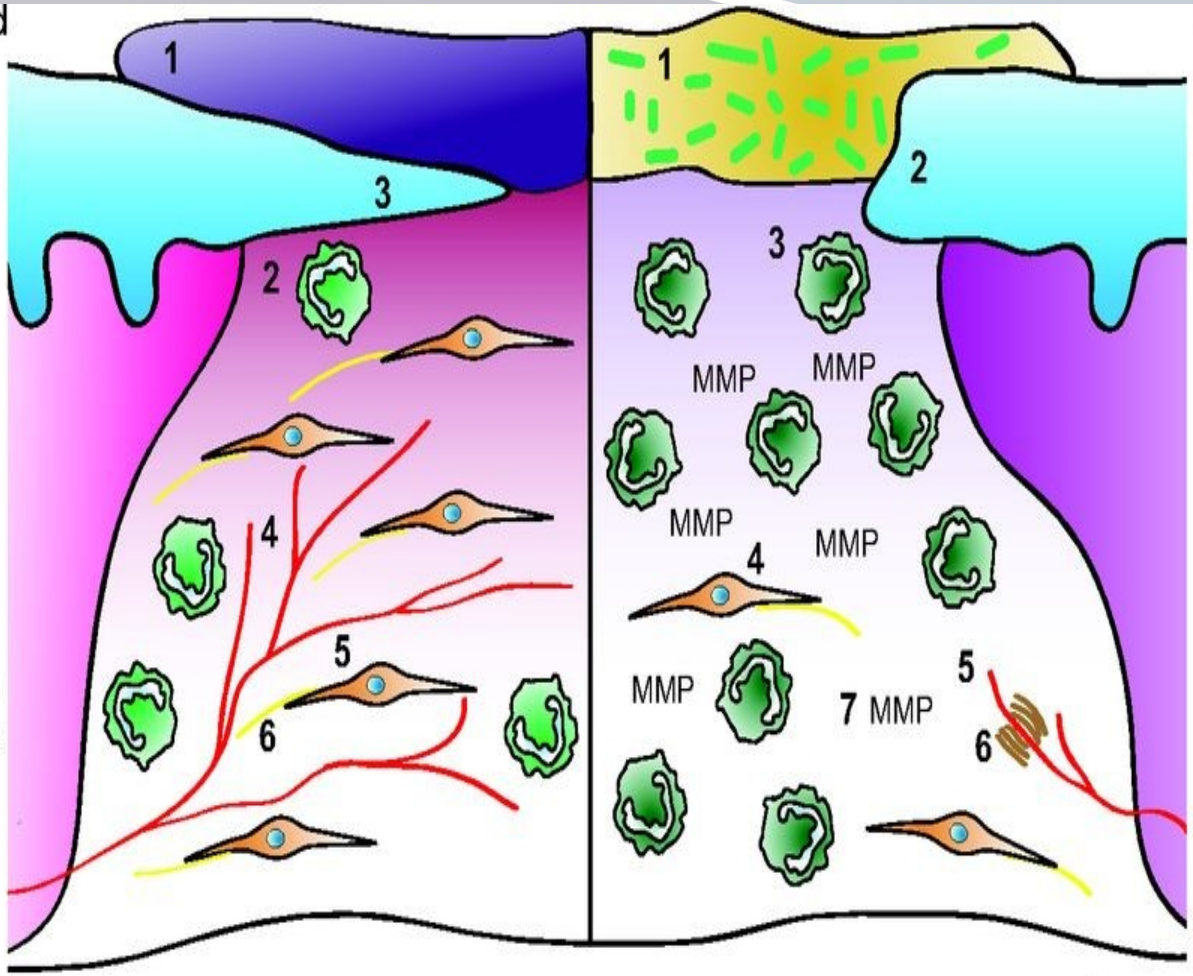
Acute (healing) wound

Inital phase:

1. Scab formation
2. Immune cell infiltration

Healing phase:

3. Re-epithelialisation
4. Angiogenesis
5. Fibroblast migration
6. Collagen deposition

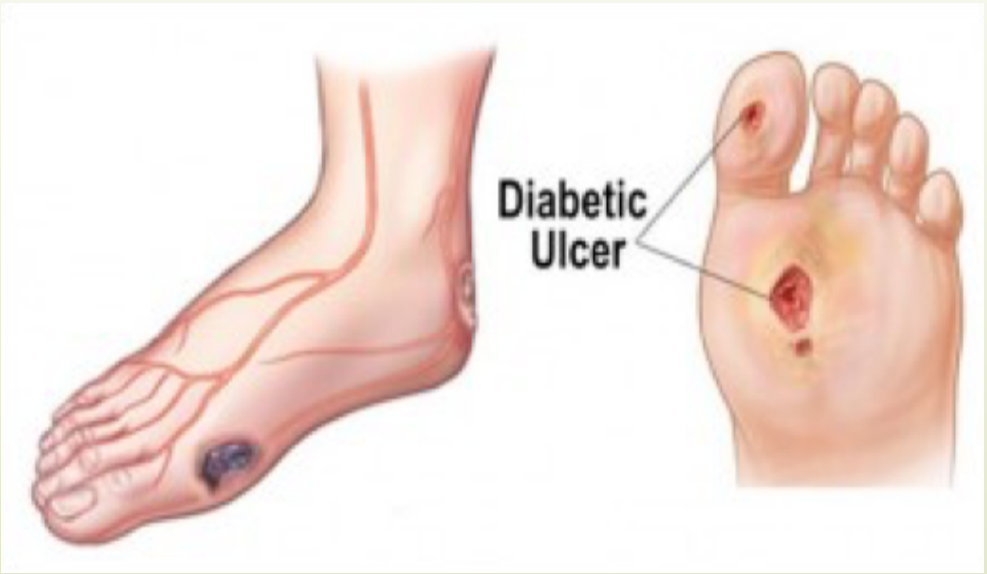


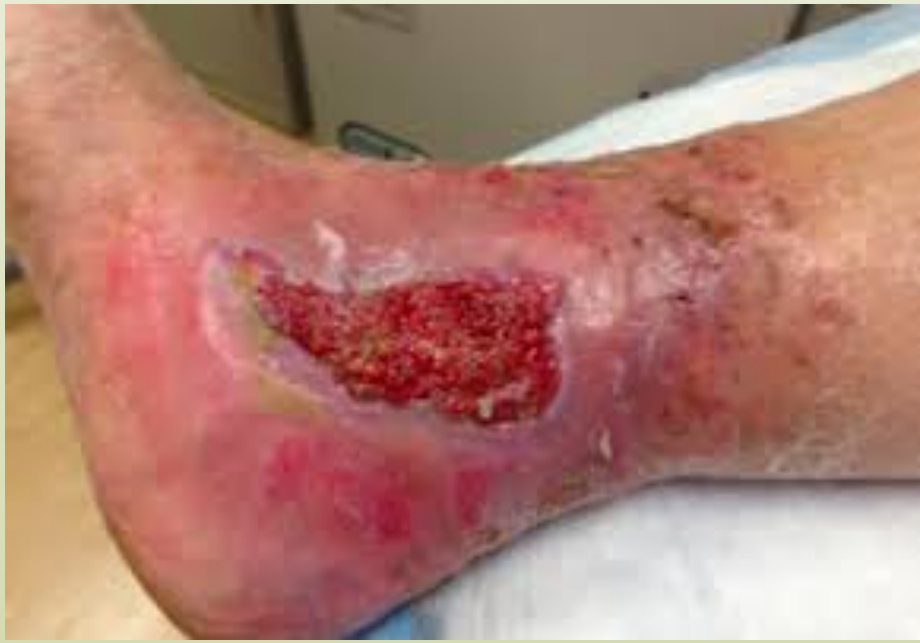
Chronic (non-healing) wound

Chronic wound abnormalities:

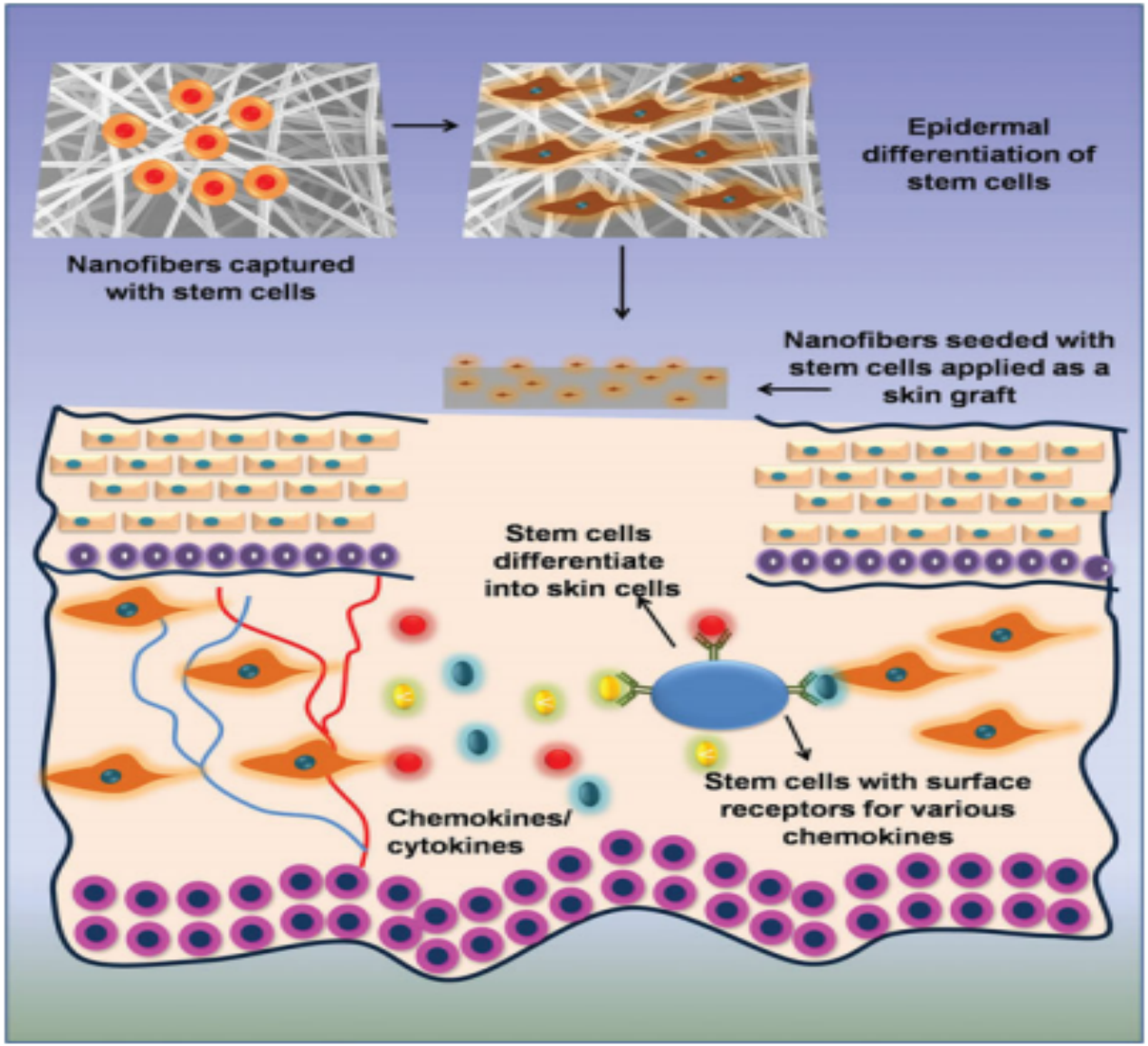
1. Infection/biofilm
2. Hyperproliferative epidermis/ stalled re-epithelialisation
3. Persistent inflammation
4. Fibroblast senescence
5. Impaired angiogenesis
6. Fibrin cuffs (barrier to oxygen)
7. Elevated MMPs

Diabetic Foot Ulcers (Chronic Wounds)





Skin Tissue Engineering



Skin Tissue Engineering

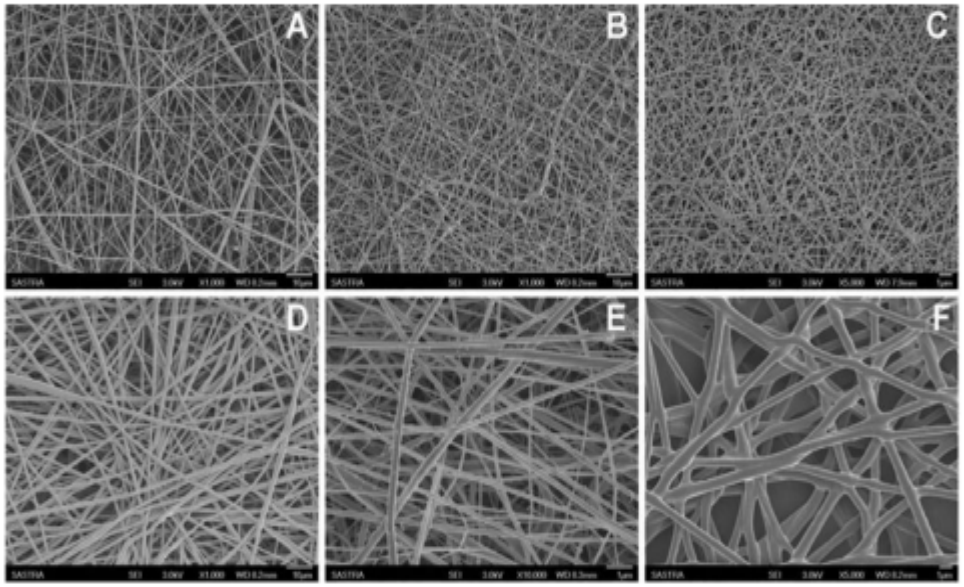


Figure 5. Different types of polymeric nanofibrous scaffolds investigated for skin tissue engineering applications. [A] Poly(1,4 butylene succinate) (PBSu); [B] PHBV-PBSu; [C] Chitosan-PVA; [D] PLGA; [E] PLA; [F] PHBV.

Table 1

Polymeric nanofibers investigated for skin tissue engineering applications

Nanofibrous Material	Study	Inference	Ref
Collagen	Implanted in full-thickness wound in athymic mice	Reduce wound contraction. Skin substitute for full thickness burns	47
Gelatin	*In vitro* using human skin fibroblasts	Potential dermal-epidermal skin substitute. High cell infiltration suitable for dermal-epidermal skin substitute	48
Silk fibroin	*In vitro* using normal human keratinocytes and fibroblasts	Cytocompatible for human keratinocytes and fibroblasts. Good wound dressing material	49
Myoglobin and hemoglobin	Morphological characterization	Can prevent wound hypoxia and promote healing	50
Poly(3–hydroxybutyrate–*co*–3–hydroxy valerate) (PHBV)	*In vitro* using human skin fibroblasts and *in vivo* in rat model	Promotes cell proliferation and topical administration of R-Spondin 1 enhances angiogenesis *in vivo*	16
Poly(lactide–*co*–glycolide) (PLGA)	*In vitro* using normal human keratinocytes	*In vitro* compatible and possess anti-adhesive property thus favors healing	9
Poly(ϵ–caprolactone) (PCL)-gelatin	*In vitro* culture on both si :s using normal human keratinocytes	3-D dermal substitute with enhanced cell infiltration for accelerated dermal wound healing	51
Chitosan grafted PCL/PCL (Polycaprolactone)	Cell-scaffold interaction by culturing mouse fibroblast cells	Cationic nanofibers promotes cell attachment and proliferation	52
Plasma-treated PLACL(poly(L–lactic acid)–*co*–poly (ε–caprolactone)) / gelatin	*In vitro* using human foreskin fibroblast	Promotes cell proliferation and collagen expression	53
PVA-PHB (polyvinyl alcohol and polyhydroxybutyrate)	*In vitro* using HaCaT (Keratinocytes) and fibroblast cells	Supports HaCaT and fibroblast proliferation	54
Chitosan	Implanted in third degree	Enables exudates	55

Advantages and disadvantages of synthetic polymers in skin tissue engineering

S.No	Polymers	Advantages	Disadvantages	Salient feature as skin scaffold	Ref.
1.	Poly(lactide–*co*–glycolide) (PLGA)	FDA approved, soluble in most of the organic solvents, cytocompatible	Acid degradation products	Excellent anti-adhesive property	9
2.	Poly(ϵ–caprolactone) (PCL)	FDA approved, biocompatible, good mechanical property, soluble in most of the organic solvents	Highly elastic, slow degrading	Promotes diabetic wound healing	51, 117
3.	Poly(3–hydroxy butyrate–*co*–3–hydroxyvalerate) (PHBV)	Biocompatible, oxygen permeable, biodegradable	Hydrophobic	Supports adhesion, proliferation of human fibroblasts and keratinocytes	16
4.	Polyurethane (PU)	Good mechanical strength, creates a moist environment	Highly hydrophobic, less cytocompatible	Suitable coverage for burns	56, 71
5.	Poly(L–lactide) (PLLA)	FDA approved, biocompatible, excellent cellular compatibility, soluble in most of the organic solvents	Slow degradation, mechanical stiffness, hydrophobic	Suitable for drug delivery in the wound bed	118
6.	Poly(L–lactic acid)–co–poly(ϵ–caprolactone) (PLCL)	FDA approved, good mechanical strength	Hydrophobic, less cytocompatible	Suitable for encapsulating wound healing mediators/growth factors	119

Growth factor conjugated nanofibrous scaffolds used in skin tissue engineering.

Scaffold	Growth factor conjugated	Study	Inference	Ref
Activated platelet rich plasma (Blend electrospinning)	FGF, VEGF, EGF	*In vitro*	Rapid cellular infiltration	77
PCL-PEG/PCL poly(ε-caprolactone)-poly(ethyleneglycol)/poly(ε-caprolactone) (Chemical conjugation)	EGF	*In vitro* and *in vivo* diabetic mice	Promotes keratinocytes differentiation and significant wound closure	81
PELA (PEG-PLA) Poly(ethylene oxide-*co*-lactic acid) (Core-sheath nanofibers)	bFGF	Tested *in vitro* and *in vivo* in diabetic rats	Sustained release of bFGF resulted in complete epithelialization after 4 weeks	78
PLCL (Poly(L–lactic acid)–*co*–poly (ε–caprolactone)) (Core-shell nanofibers)	Encapsulated with EGF, Insulin, hydrocorti-sone and retinoic acid	*In vitro* differenti-ation of Adipose derived stem cells (ADSCs)	Sustained release of factors enabled epidermal differentiation of ADSCs	79