

PART M.SCHÄFER

WOUND HEALING

WOUND HEALING AND SKIN FUNCTION

Why wound healing?

- **Important medical problem**
 - o Chronic wounds/ulcers (patients with diabetes, immunosuppression, chemo-, radiotherapy)
 - o Hypertrophic scars and keloids
- **Partial recapitulation of developmental processes**
- **Important for cancer research**
 - o „Tumors are wounds that do not heal“

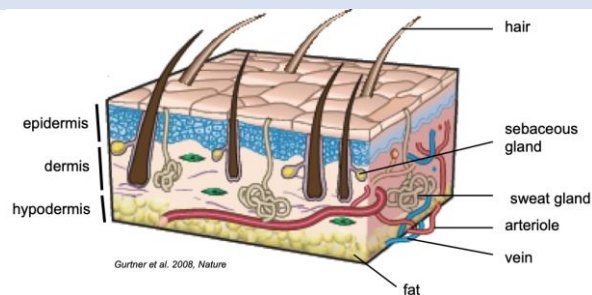
BACKGROUND INFO (DON'T LEARN):

- **Chronic wound** (→ long healing process)
 - o Wound often remains in inflammatory phase
 - o Very painful
 - o High medical costs
 - o High risk of infection
 - o High risk of malignant transformation
- **Keloid:**
 - o Benign fibrotic tumor
 - o Genetic predisposition
 - o Sometimes painful
 - o Excessive collagen deposition (type I, III)

FUNCTION OF THE SKIN:

- **Protection** (UV, toxins, chemicals, irradiation, pathogens, physical insults, ...)
- **Regulation** of the body **temperature**
- **Barrier** against **water loss**
- **Sensory** organ (pain, temperature, pressure,...)

SKIN ORGANISATION



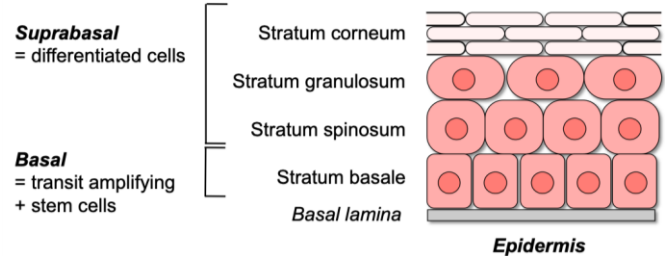
The skin consists of **three layers**:

- **Epidermis:** Upper/outermost layer, Keratinocytes (*Barrier function*)
- **Dermis:** Mechanically stable and elastic
 - o Fibroblasts and extracellular matrix: *Flexibility*
 - o **Sweat glands:** *Thermoregulation*
 - o **Hair with sebaceous gland:** *Thermoregulation, protection (absorption of UV light), lipid secretion for water repulsion*
- **Hypodermis:**
 - o Adipose tissue: *„polster“, energy store, thermoisolation*
 - o Arteriole, vein: *Provision of nutrient, Thermoregulation, Important for wound healing*
- **Muscle** under hypodermis

EPIDERMIS

LAYERS OF THE EPIDERMIS

- **Stratum corneum:** *Tightly anchored, Flat keratinocytes w/o nucleus (not dead, are metabolically active) for water barrier formation by corneodesmosomes and cornified cell envelope*
- **Stratum granulosum:** *Cells containing lamellar bodies filled with lipids, tight junctions as a second barrier*
- **Stratum spinosum**
- **Stratum basale:** *Stem cell and transient amplifying cells*
- **Basal lamina:** *Barrier towards underlying tissue*



DIFFERENT CELL TYPES:

- **Keratinocytes:** Formation of a barrier against environmental damage
 - o Undergo tightly controlled differentiation program
 - o Stainings: H&E Staining, K10 & K14, Loricrin)
- **Merkel cells:** Receptor cells connected to somatosensory nerve fibers, neuroendocrine function
- **Immune cells** (e.g. Langerhans cells, T-cells)
- **Melanocytes:** Produce melanin and thereby protect cells from UV damage
 - o Melanin accumulates on top of the nuclei

STEM CELLS

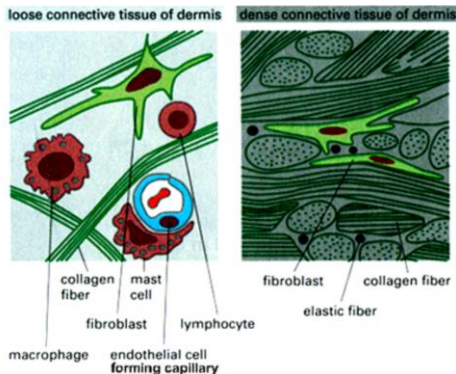
- Stem cells in **stratum basale**
 - o Not on the surface due to UV protection
 - o As soon as stem cells move up, they start to differentiate → they start to express different markers and differentiate into different cell layers

How is the epidermal renewal regulated in the stratum basale?

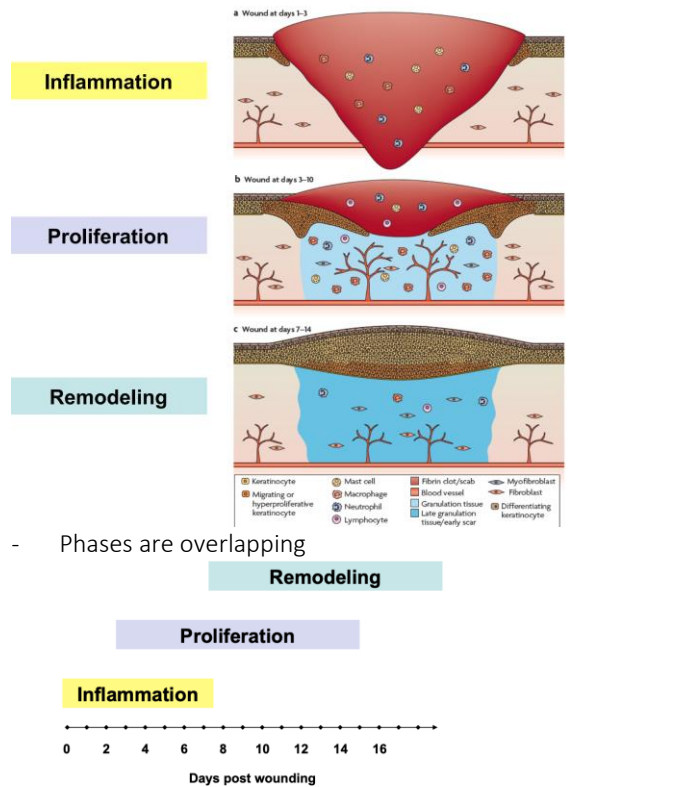
1. Stem cells bind to the basal lamina
 2. Stem cell daughter turn into transient amplifying cells (TAM) → high proliferative
 3. The newly generated cells migrate to the upper layer
 4. Differentiation mediated by pH and calcium changes
- Constant renewal with shedding at the end: Because of UV

DERMIS

- Consists of loose and dense **connective tissue**
 - o Loose connective tissue
 - o Dense connective tissue
- **Fibroblasts:** Synthesize extracellular matrix and collagen
- **Immune cells** (e.g. T-cells, Macrophages, Neutrophils)
- **Endothelial cells:** Form the endothelium (interior surface of blood vessels and lymphatic vessels)
- **Cutaneous Nerves:** (also in epidermis) Sensory innervation
- **Smooth muscle cells:** Form erector pili muscle and blood/lymphatic vessels



3 PHASES OF WOUND HEALING



1. PHASE: INFLAMMATION PHASE (1-3 DAYS)

1. Blood clot formation
2. Invasion of immune cells

1. BLOOD CLOT FORMATION



1. Damage of blood vessels
 - o Platelets bind to interstitial connective tissue
 - o Immune cells come with blood
2. Aggregation of platelets
 - o Degranulation
 - o Release of growth factors and chemotactic factor for neutrophils/macrophages as well as for resident cells

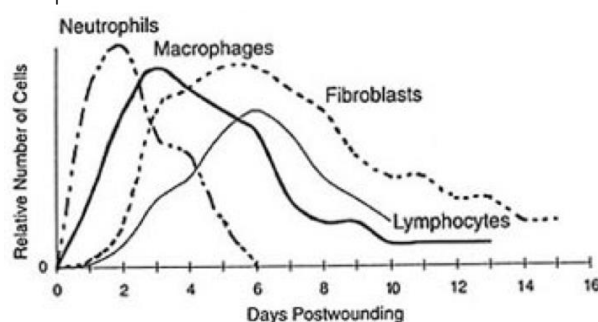
More and more platelets binds → plug, binding activates → degranulation → Growth factors → more immune cells
3. Blood coagulation
 - o Formation of fibrin clot
 - Plugs wound → Serves as provisional matrix

Function of Blood Clot:

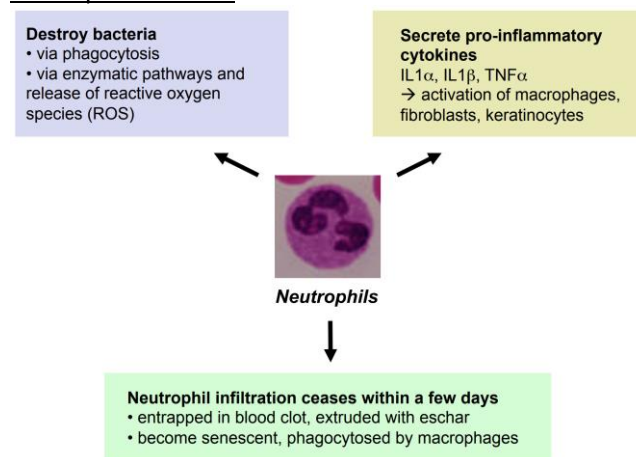
- Protection of wound tissue (invading microorganisms, water loss)
- Matrix for migrating cells
- Reservoir of cytokines and growth factors
 - o Recruitment of inflammatory cells
 - o Initiation of re-epithelialization
 - o Formation of the granulation tissue
 - o Stimulation of connective tissue contraction

2) INVASION OF IMMUNE CELLS

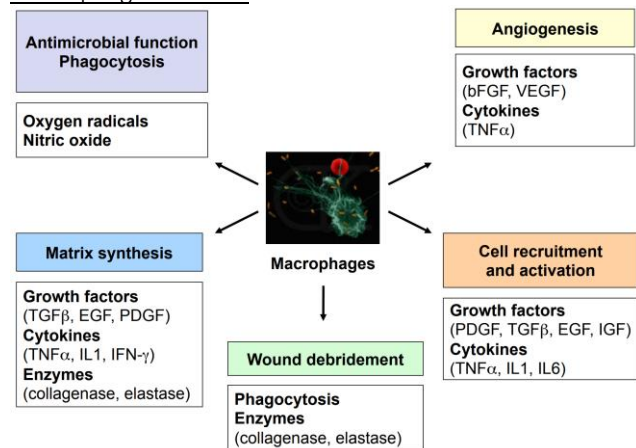
- Facilitated by vasodilation and enhance vascular permeability
- Neutrophils and monocytes migrate concurrently into the wound
- Sequence in invasion:
 - o Neutrophils → macrophages → fibroblasts → lymphocytes
- Neutrophils arrive first in large number due to their higher abundance in the circulation
- ROS production in the wound



Neutrophil functions:



Macrophage function:



2. PHASE: PROLIFERATION PHASE (3-15 DAYS)

1. Re-epithelialization

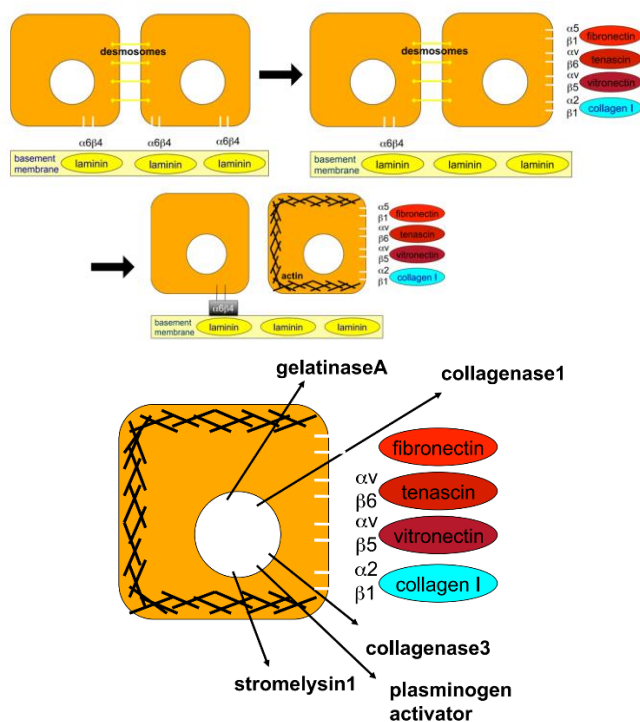
2. Formation of Granulation tissue

- Neovascularization
- Fibroblast migration and proliferation
- Matrix deposition
- Wound contraction

1. RE-EPITHELIALIZATION

= Keratinocyte migration and proliferation

- Stimuli:
 - o "Free edge" effect → Absence of neighboring cells (every keratinocyte needs neighbors around)
 - o High concentration of growth factors
 - EGF family (EGF, HB-EGF, TGFα), HGF, KGF
- 1. No keratin connection towards the side of the blood clot, but extracellular matrix proteins → Fibronectin, tenascin, vitronectin und collagen 1 attach to keratinocyte
- 2. Migration is triggered: Desmosomal connections to remaining neighbor & connection to basal lamina is lost
- 3. Actin remodeling → Migration towards blood clot (Accumulation of actin)
- 4. Secretion of proteases to get through the ECM (gelatinase A, collagenase 1/3, plasminogen activator, stromelysin1)

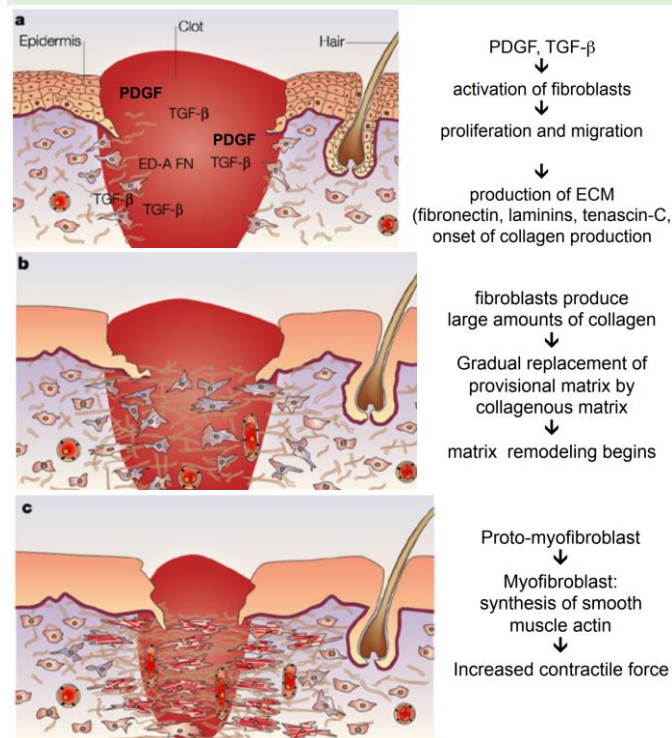


2. FORMATION OF GRANULATION TISSUE

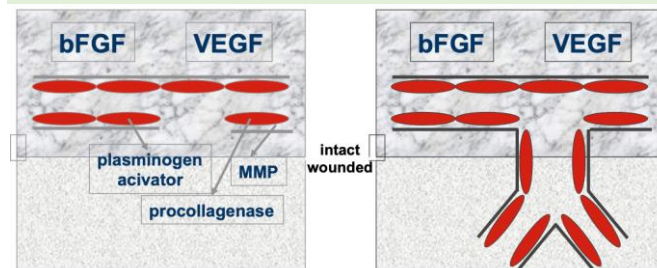
- Fibroblast migration and proliferation:

- PDGF, TGF-β secretion from macrophages → Activation of fibroblasts → Proliferation and migration → Production of ECM (fibronectin, laminins, tenascin-C, onset of collagen production) → Fibroblasts produce large amounts of collagen → Gradual replacement of provisional matrix by collagenous matrix (matrix deposition) → Matrix remodeling begins

1. Neurovascularization
2. Fibroblast migration and proliferation
3. Matrix deposition
4. Wound contraction



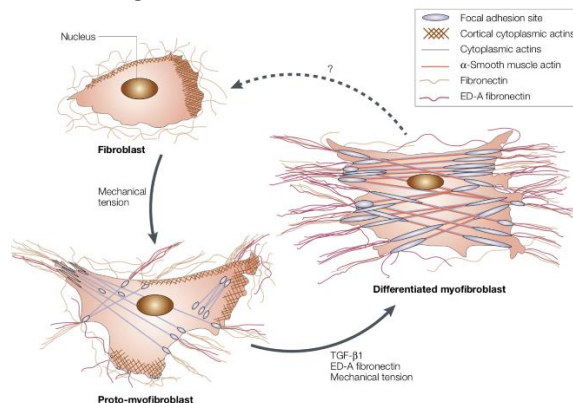
- Neovascularization



1. bFGF and VEGF secretion
2. Proliferation and migration of endothelial cells
3. Plasminogen activator, MMP and procollagenase secreted → enable ECM deposition in wounded area
4. Formation of new basement membrane around vessels

- Wound contraction

1. Fibroblast + mechanical tension → proto-myofibroblast
 2. Proto-myofibroblast + TGF-β/ED-A fibronectin + mechanical tension → differentiated myofibroblast
 3. Myofibroblast: Synthesis of smooth muscle actin → Increased contractile force
- Further: Migration of cells into wound increases tension



3. PHASE: REMODELING PHASE (7-18 DAYS)

1. Epithelium → Epidermis
2. Granulation tissue → Dermis

Remodeling

= Transition from granulation tissue to mature scar

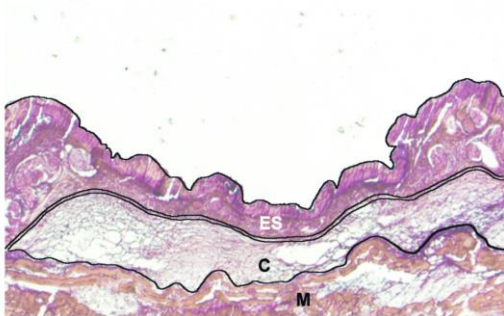
1. Apoptosis of fibroblasts/myofibroblasts
2. Regression of capillaries
3. Collagen remodeling
 - o Collagen synthesis by fibroblasts
 - o Collagen catabolism: Tightly regulated process involving metalloproteinases and their inhibitors formation of larger collagen bundles, alterations of intermolecular cross-links
 - o Formation of larger collagen bundles
 - o Alterations of intermolecular crosslinks

OUTCOME OF THE WOUND HEALING PROCESS

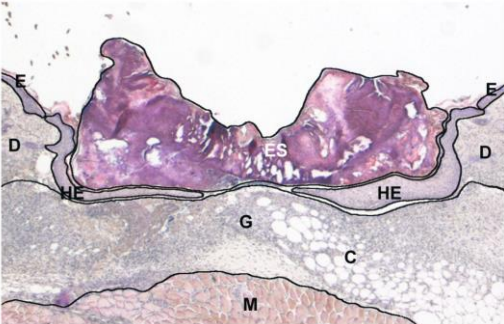
- Healed skin
 - o neither aesthetically nor functionally perfect
- Loss of skin appendages (hair follicles, sweat glands, sebaceous glands)
- Reduced tensile strength
 - o Within first 3 weeks after injury: 20% of the final tensile strength
 - o Mature scar: max. 70% of tensile strength of uninjured skin

WOUND PICTURES OF MOUSE BACK SKIN

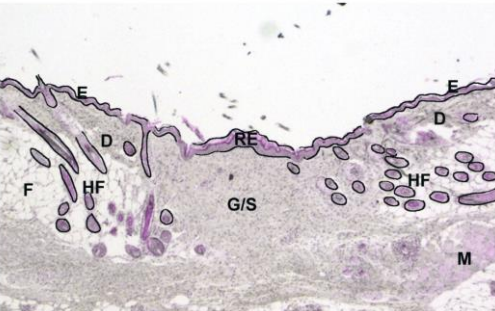
a 1-day wound



b 5-day wound



c 14-day wound



FETAL WOUND HEALING

- Cellular:
 - o Fast re-epithelialization
 - o Fetal fibroblasts (No myofibroblasts but actin cables in the wound)
 - o Low inflammation
 - o Nerve regeneration
 - Molecular:
 - o Low levels of TGFβ1
 - o High levels of hyaluronic acid
 - o High levels of MMPs
- No scarring (until third trimester)
- Different matrix reorganization
 - Remodeling functions are better without infection risk

WOUND HEALING IN PATIENTS WITH DIABETES

BACKGROUND INFORMATION (DON'T LEARN)

Chronic wound:

1. Venous leg ulcers:

- venous hypertension (improper function of valves)
- lower pressure in arteries compared to veins
- ischemia, reperfusion
- inflammation, ROS, assembly of immune cells in small vessels

2. Diabetic foot ulcer:

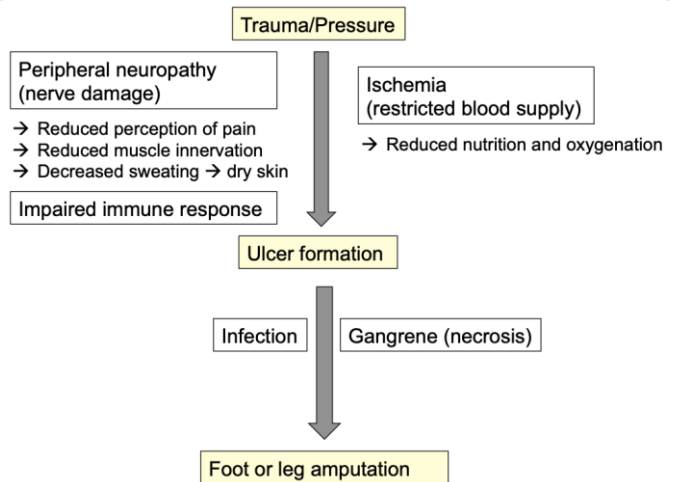
- neuropathy
- wound infection, immune compromised, defective small vessels
- Hypoxia
- High risk of amputation

3. Pressure ulcers:

- e.g. patients paralysis
- restricted blood flow (pressure higher than in vessels)
- Malnutrition

DIABETIC FOOT ULCER

= Big wound reaching the fat tissue



HISTOPATHOLOGICAL FEATURES:

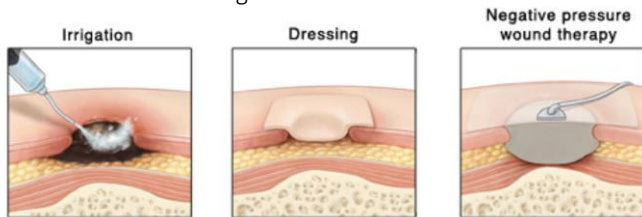
- Reduced migration and proliferation of keratinocytes and fibroblasts
- Delay in mature granulation tissue formation
- Reduced Angiogenesis
- Disturbed extracellular matrix formation and remodeling (byupregulation of MMPs)
- Reduced innervation (Neuropathy, nerve formation)

MOLECULAR CHANGES:

- **Fibroblasts:** Senescent and reduced response to growth factors
- **Macrophages:** Reduced secretion of cytokines (IL1 β , VEGF)
- **Growth factors:** Trapped and reduced expression, degradation of growth factors and their receptors by MMPs
 → Reduced cell migration, proliferation and attraction
- **MMPs:** Excessive activation
 → Impaired cell migration, degradation of matrix proteins and growth factors
- 4. **Nitric oxide:** Reduced levels
 → Reduced fibroblast proliferation
 → Reduced collagen production
 → Reduced angiogenesis

STANDARD THERAPY

- Removal of excessive cells and fluid
 → Wound debridement (surgical removal of tissue)
 → Negative pressure
- Treatment of infection
- Correction of perfusion/ oxygenation
 → Hyperbaric oxygen therapy
 → Negative pressure
- Enhancement of wound closure
 → Wound dressing



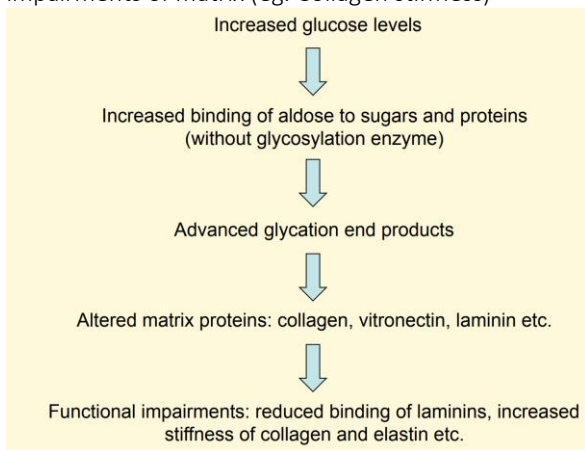
CLINICAL TRIALS

Application of:

- Growth factors (PDGF-BB)
- Extracellular matrix proteins (collagen, hyaluronic acid)
- Bioengineered skin
- Cultured autologous bone-marrow cells

POTENTIAL RELATION TO DIABETES IN FOOT ULCERS

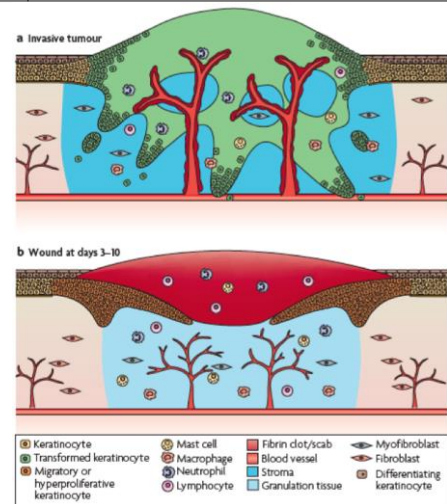
Increased glucose levels → Advanced glycation in the end products → Altered matrix proteins → Functional impairments of matrix (eg. Collagen stiffness)



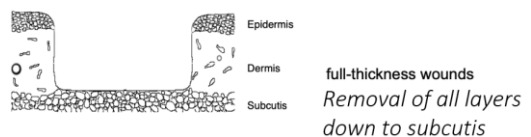
Background information

PARALLELS: WOUND HEALING AND CANCER

	Wound	Cancer
Fibrin matrix	Blood clot formation (damaged blood vessels)	Chronic fibrin deposition (hyperpermeability of vessels)
Inflammation	transient	persistent → protumorigenic → stimulates angiogenesis + ECM breakdown → enhances cancer cell motility + invasion → promotes malignancy (ROS, NOS)
Epithelial Proliferation + Migration	transient	persistent
Epithelial-mesenchymal transition (EMT)	partial → Remaining intercellular junctions + keratin expression	complete (metastasis) → Loss of cell-cell contacts → Fibroblast like morphology → Expression mesenchymal markers
Stimulation: HGF, TGF β , TNF α , MMPs, <i>only tumor</i> : Ras mutations		
	Wound	Cancer
Fibrous tissue	Granulation tissue → fibrous tissue	Persistent Stroma formation Microenvironment: → tumor progression → cancer cell invasion
Fibroblast activation: PDGF, TGF β and others		
Angiogenesis	transient	Persistent + imperfect → essential for tumor growth
Stimulation: VEGFA, PLGF, FGF2 and others Inhibition: TSP1, IP10		



MICE: MODEL ORGANISM FOR WOUND HEALING



- Require:
 - o Appropriate animal facilities
 - o Training (in house and official courses)
 - o Application and permission Regular reports
- Control mechanisms:
 - o Written application
 - o Control of reports
 - o Unannounced controls in the lab
- Experiment:
 - o Anaesthesia, including pain control
 - o Shaving, surgery
 - o Daily control