BIOMEDICÍNSKÉ CENTRUM

LÉKAŘSKÁ FAKULTA V PLZNI
UNIVERZITA KARLOVA V PRAZE

Pathophysiology of wound healing

Pavel Klein, Ph.D.

pavel.klein@lfp.cuni.cz

EVROPSKÁ UNIE
EVROPSKÝ FOND PRO REGIONÁLNÍ ROZVOJ
INVESTICE DO VAŠÍ BUDOUCNOSTI

OP Výzkum a vývoj pro inovace

Classification of wounds

There may be more classification systems. For example, the following aspects are important:

1) Clinical aspect - each type of wound needs different treatment
2) Forensic point of view - a large number of wounds arise as a result of crime
3) Preventive safety aspect - a significant number of wounds arise as a result of non-observance of safety rules (at work, sports, etc.)

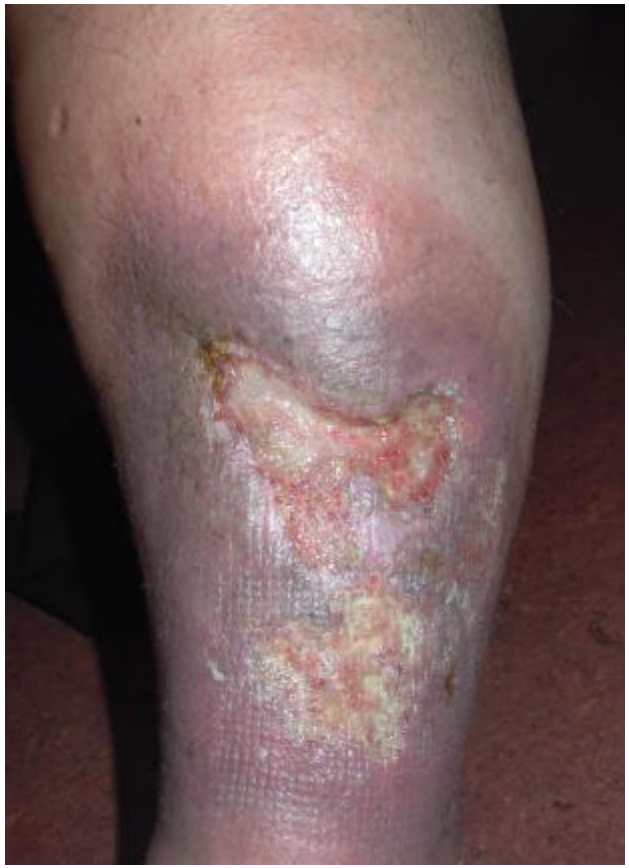
From the clinical point of view, the wounds can be divided according to:

1) speed of healing
2) the way of healing
3) etiology

Classification of wounds

1) Classification according to the speed of healing

A. Acute
B. Chronic

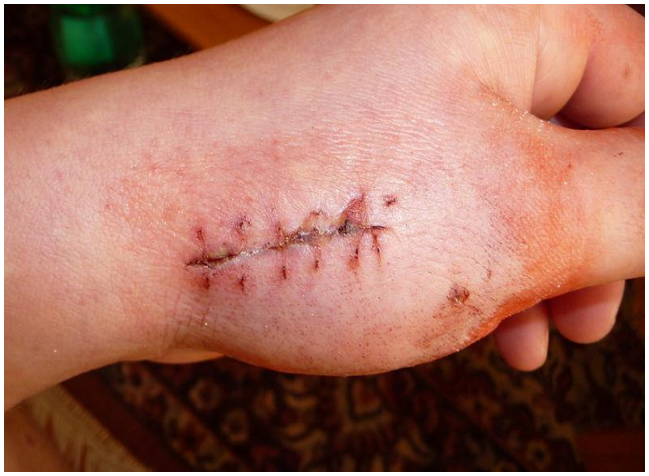


Ulcus cruris, leg ulcer. Typical example of the chronic wound

Classification of wounds

2) Classification according to the way of healing

A. Wound healing *per primam intentionem* = for the first time, without complications. These wounds are clean, uninfected, mostly with smooth margins

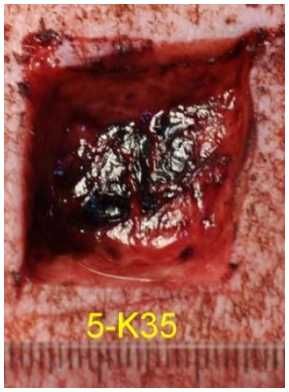


About 10 days old cut wound treated with suture and healed *per primam intentionem*

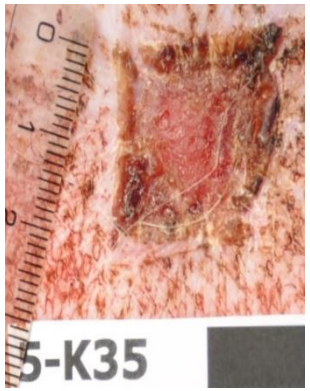
Classification of wounds

2) Classification according to the way of healing

B. Wound healing *per secundam intentionem*, for the second time - wounds infected and / or widely open. The healing process itself is preceded by acute inflammation and then (if the healing proces is physiological) the defect is gradually filled with granulation tissue.



Experimental wound in a pig
3 days after the injury



The same wound 10 days later

C. Wound healing for the third time or *per tertiam intentionem* - usually deep wounds that were not initially closed by suture (e.g. opened to the débridement) and which were allowed to heal *per secundam intentionem*. Subsequently (for aesthetic reasons, for example) the healing process is purposefully disrupted and the wound is treated with suture, insertion of a skin graft, etc.

Classification of wounds

3) Classification according to etiology

A. Wounds caused by external mechanical, physical and chemical factors

B. Wounds resulting from pathological changes or external factors on pathologically altered tissue (diabetic wounds, leg ulcers, etc.)

Classification of wounds

3) According to etiology

Brief overview of wounds caused by external factors

Mechanical	**Thermal**	**Chemical**	**Radiation**
Cutting wound	Burn	Caustic burn	skin damage by UV radiation
Stab wound	Frostbite		skin damage by ionizing radiation
Wound from edged weapon			
Laceration wound			
Bruised laceration wound			
Gunshot wounds			
Abrasion			
Bite wound			
Surgical wound			

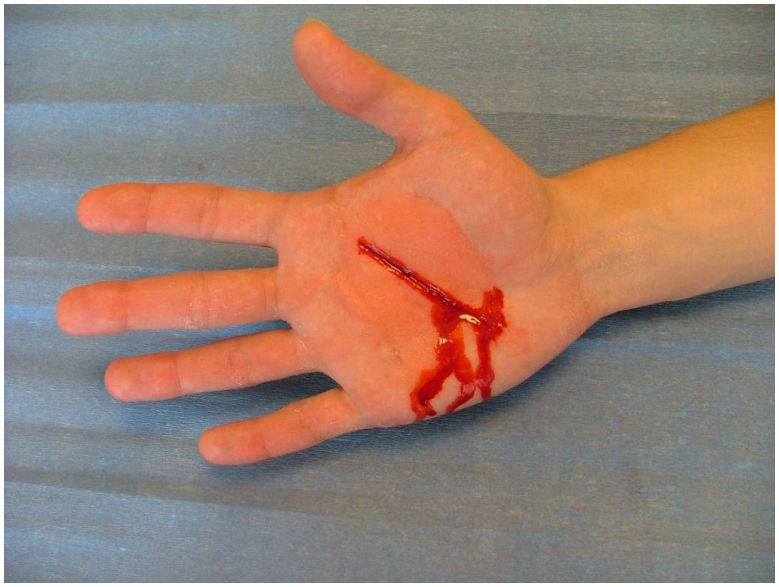
Classification of wounds

I. Cutting wound (*incisio*)

The cause is sharp edge injury (knife, piece of glass...)

Nature of wound: bleeding from sharp edges and damaged blood vessels, minimal or no tissue loss; however, blood loss and shock symptoms may occur depending on the extent

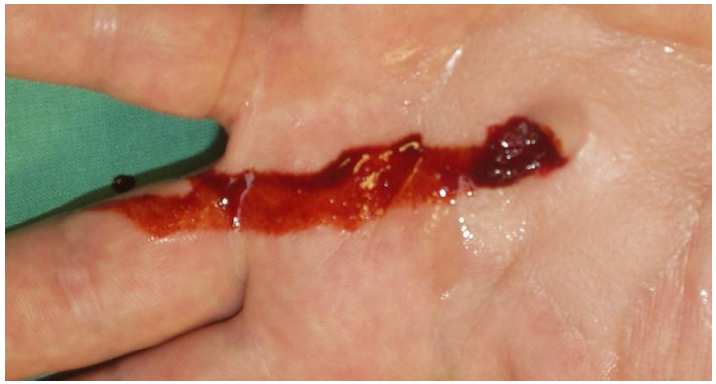
The treatment depends on the extent of the injury and the structures affected. The most serious complication is the injury of large blood vessels. Depending on the extent, this wound is usually treated with suture. Healing in uncomplicated courses is usually *per primam intentionem*



Classification of wounds

II. Stab wound (*vulnus punctum*)

The cause is to stab a longer sharp object into the tissue (knife, nail, shard, stabbing weapon...)



Wound nature: minimal or no tissue loss; a stab wound usually does not bleed massively (lower probability of intervention of larger vessels). However, depending on location and depth, vital organs may be affected

The treatment depends on the extent and especially the depth of the injury and the structures affected. The most serious complication is stuck body in the wound - do not pull out!
Healing of stab wounds is usually not complicated.
Higher risk of tetanus disease !!

Classification of wounds

III. Wound from edged weapon (*vulnus sectum*)

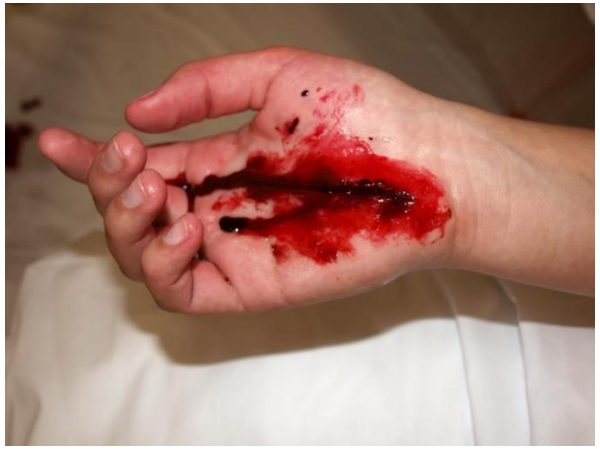
Causes: This wound is caused by the impact of a sharp object (ax, cutting weapon, sharp plate, etc.) on the body surface.

Character of the wound: very similar to the cut wound, but rather wedge-shaped, deeper, possibly tissue loss.

Treatment and healing depend on the extent and especially the depth of the injury and the affected structures, which may be, for example, bone, including the skull. Otherwise, the procedure is similar to the cutting wounds

Classification of wounds

IV. Laceration (*vulnus lacerum*)



The cause is tissue tearing due to tension. Depending on the application of forces, it may be purely lacerated or bruised and lacerated.

Wound character: similar to cutting wounds, but the edges are wavy, irregular wound shape. They are not usually deep. There may be tissue loss.

The treatment consists mainly of suture.

Healing of lacerations is more complicated than in cutting wound healing and, depending on the extent of tissue loss, it also heals *per secundam*. The greatest complication is infection

Classification of wounds

V. Gunshot wounds (*vulnus sclopetarium*)

The cause is a bullet penetration or shrapnel penetration. A specific category is then shotgun shot.

We distinguish: A thorough damage – it has entrance and exit apertures (a bullet is out of the organism).
Blind injury – in has only entrance aperture (bullet is at the end of wound canal). Tangential – the damage of superficial tissues, without the penetration to the cavities of the organism.

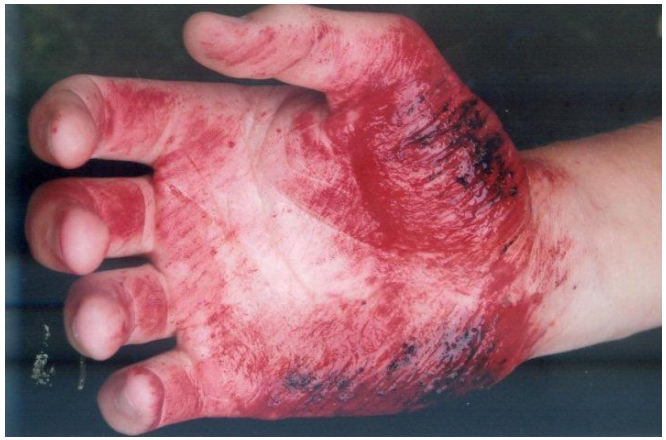
Treatment: surgery almost always required. The shots bleed less, but there is a much higher risk of internal bleeding

Healing is usually complicated. Wounds are almost always contaminated (bacteria, barrel oil, gunpowder, metal particles, etc.). Due to the considerable kinetic energy, there is a mechanical effect on the internal organs. Gunshot wounds usually heal with a scar.

Classification of wounds

VI. Abrasion (*abrasio*)

This is caused by the effect of the coarse material on the skin surface



Character of the wound: Abrasions are flat shallow wounds affecting the epidermis or part of the dermis. They are accompanied by tissue loss, less bleeding (only the capillary network is broken), and very painful, especially if they are accompanied by contusion. They are often contaminated with particles (slag, dust, sand...)

Treatment: mechanical cleaning of the wound is almost always necessary (risk of traumatic tattooing!). Wet treatment is suitable.

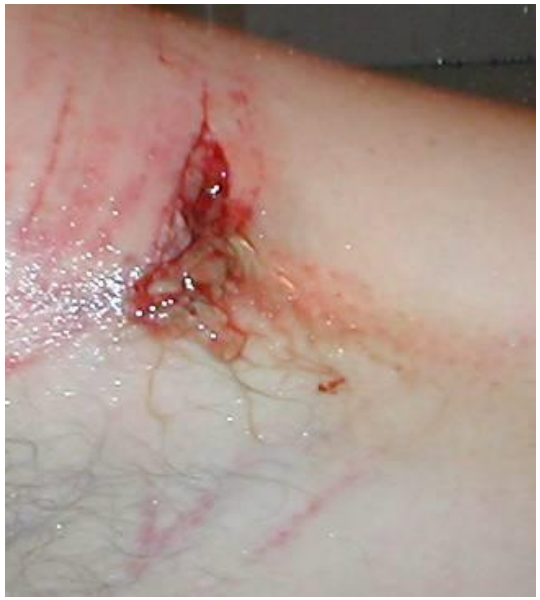
Healing of abrasions is usually without complications when properly treated. Although, it is necessary to restore the epidermis over a large area, it often heals without scars

Classification of wounds

VII. Bite wound (*Vulnus morsum*)

This is caused by an animal or human bite

Wound character: it depends on the strength of the push and the way the attack is carried out: it is most often a bruised, stab or laceration, it can be accompanied by tissue loss. Very painful



Treatment: Bites are almost always heavily contaminated wounds and therefore thorough disinfection of the wound is necessary. Wounds (unless it is a longer laceration) are usually not treated with suture and are allowed to heal per secundam with a scar.

Healing is almost always very complicated

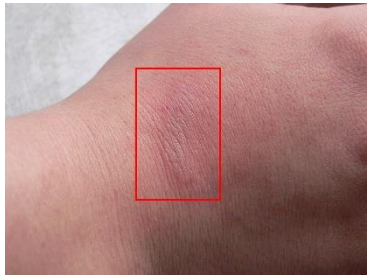
Classification of wounds

VIII. Burn

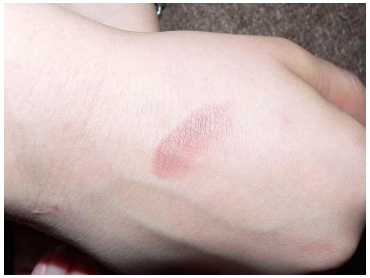
Burns are among the most serious injuries. They are caused by the effects of high temperatures; according to the extent of tissue damage, there are 4 grades:

Grade I: the skin is red, swollen, damaged skin peeling off, heals completely, without scars

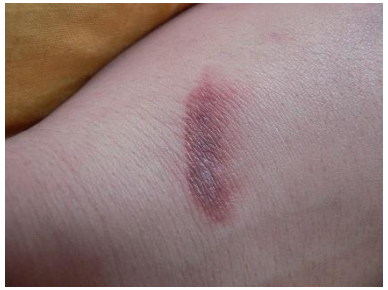
Grade II: according to the affected layers we distinguish sub-grades: **IIa** - only the epidermis and the upper part of the dermis are affected, a thin-walled blister is formed. **IIb**: Reticular layer of the dermis affected, higher risk of scarring. It heals in weeks



Burn of the grade II after 1 hour



Burn of the grade II after 24 hours



Burn of the grade II after 48 hours

Classification of wounds

VIII. Burn

Grade III: the skin is white, painless, because the nerve endings are destroyed. The tissue is irreversibly damaged, to a lesser extent it can heal by contraction, if a larger area of the skin is burned, it is necessary to insert a skin graft.

Grade IV: the tissue is charred (the skin is completely destroyed)

Classification of wounds

VIX. Frostbite

Tissue damage due to exposure to low temperatures. Risk not only in winter but also when handling dry ice or liquefied gases! The most endangered parts of the body are the fingers, ears, or even the nose

Symptoms and categorization of frostbite:

Grade 1 - the skin is waxy white or mauve, cold and insensitive. The affected person has a feeling of pinching in the place, stinging hotly during warming. Reversible state, the function is restored

Grade 2 - the skin is white to yellow, blisters may form, it is a transitional stage between reversible damage at the first stage and irreversible damage at the third stage. Sensitivity is lost at the site of frostbite.

Grade 3 - hard "waxy" skin, painless bearings, gangrene after several days.

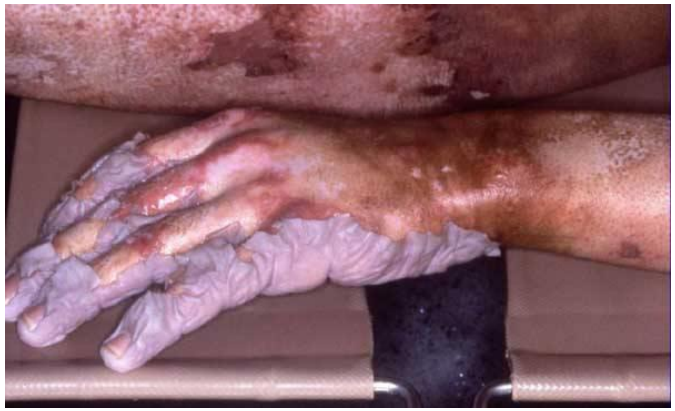
Classification of wounds

X. Caustic burns (corrosions)

Very serious, often devastating or fatal injuries, often warfare (chemical warfare agents such as mustard gas have been developed for this purpose!)

Corrosion is also caused by concentrated acids (HCl, H_2SO_4, HNO_3...) or hydroxides (KOH, NaOH). Phenol also causes severe burns

These wounds usually heal bad and long, leaving scars. The basis of the treatment is thorough removal of residues of the corrosive substance (neutralization, rinsing with water)



Hand after mustard gas attack

Classification of wounds

XI. Injury by UV radiation

It is often referred to as sunburn (symptoms are very similar to Grade I or IIa burns), but radiation damage to the tissue is predominant by UV radiation

The risk depends on the human phototype: the risk group is phototype I and II (light skin, blue / green eyes, rusty or blond hair), which always reacts with redness of the skin.

Permanent consequences: defects in pigmentation (depigmentation and pigment spots) and possibly scars. Damage to tissue by UV radiation leads to a high risk of melanoma.

Introduction

What is wound? - every tissue disruption of normal anatomic structure with consecutive loss of function.

This can range from a simple break in the epithelial integrity of the skin or it can be deeper, extending into subcutaneous tissue with damage to other structures such as tendons, muscles, vessels, nerves, parenchymal organs and even bone.

Wound healing - a highly regulated process of cellular, humoral and molecular mechanisms leading (under optimal conditions) to restoration of original structure and function.

The closure of skin can be realized by **regeneration** or **repair**. Regeneration is specific substitution of the damaged tissue, while skin repair displays an unspecific form of healing in which the wound heals by fibrosis and scar formation.

Skin regeneration – in some animals (axolotl, spiny mouse..); in humans only in fetal stadium (scarless fetal healing).

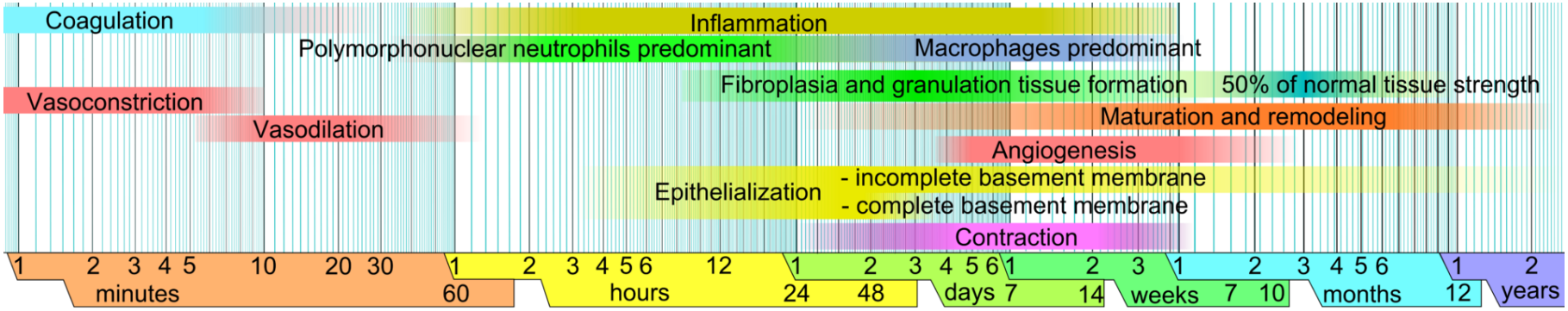
Spiny mouse

Axolotl

Introduction

The process of wound healing can artificially be divided into four phases which overlap in time and space:

1) hemostasis and coagulation
2) Inflammation
3) Proliferation
4) Maturation



Hemostasis and coagulation

vasoconstriction (5 - 10 min) triggered by the platelets, to reduce blood loss and fill the tissue gap with a blood clot comprised of cytokines and growth factors.

The vasoconstriction is then followed by a **vasodilation** in which thrombocytes invade the provisional wound matrix.

- formation of a provisional wound matrix, which occurs immediately after injury

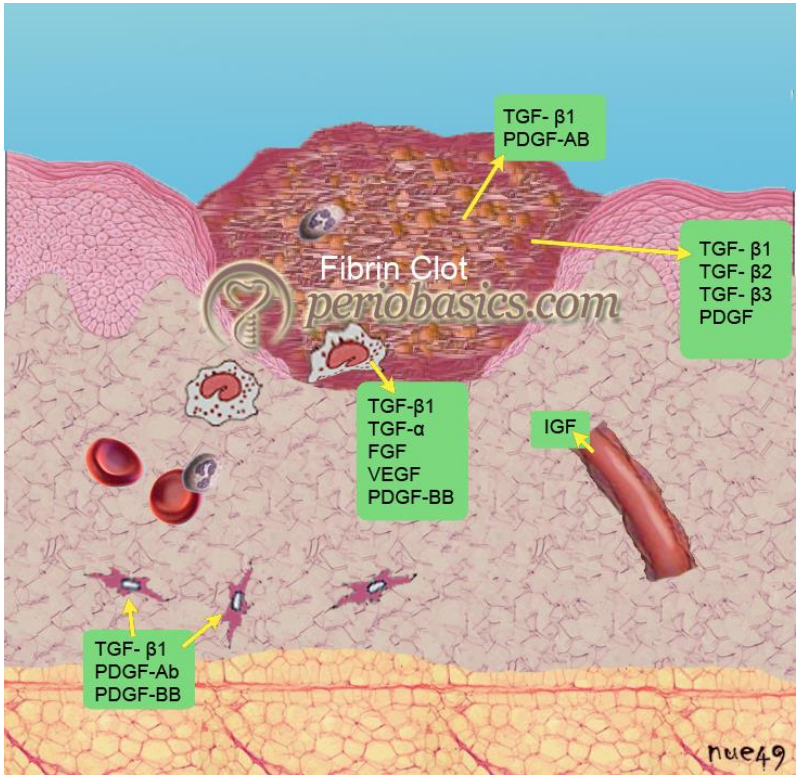
Blood clot = fibrin molecules, fibronectin, vitronectin and thrombospondins, forming the provisional matrix as a scaffold structure for the migration of leukocytes, keratinocytes, fibroblasts and endothelial cells and as a reservoir of growth factors.

- initiation of the inflammatory process and recruitment of cells and factors involved in healing.

Hemostasis and coagulation

Both thrombocytes and leukocytes **release cytokines and growth factors** to:

- activate the inflammatory process (IL-1α, IL-1β, IL-6, TNF-α)
- stimulate the collagen synthesis (FGF-2, IGF-1, TGF-β)
- activate the transformation of fibroblasts to myofibroblasts (TGF- β)
- start the angiogenesis (FGF-2, VEGF-A, HIF-1, TGF- β) and
- already support the reepithelization process (EGF, FGF-2, IGF-1, TGF- β).



Inflammation

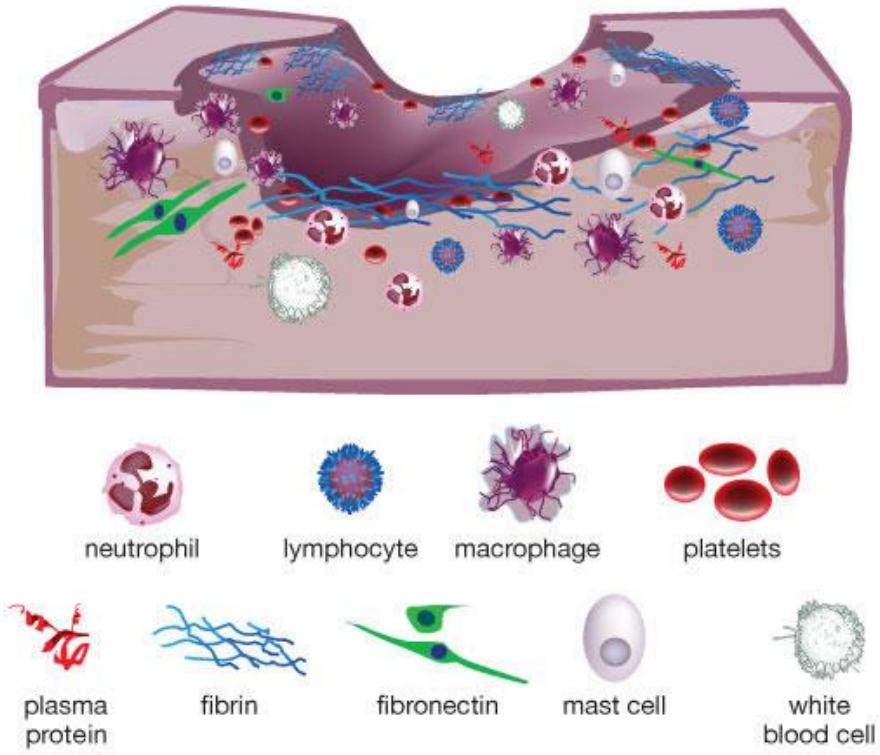
inflammation – necessary phase for killing microbial pathogens and cleaning the wound debris

3 main phases:

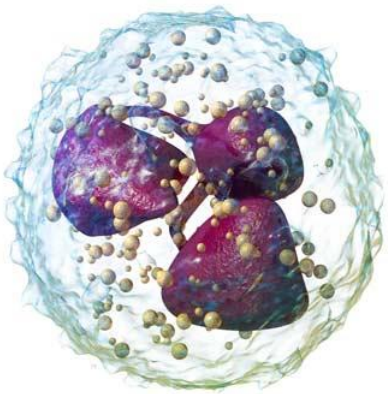
1) on the beginning: neutrophil recruitment

2) in the late phase: monocyte infiltration and differentiation to macrophage

3) lymphocyte infiltration



Inflammation

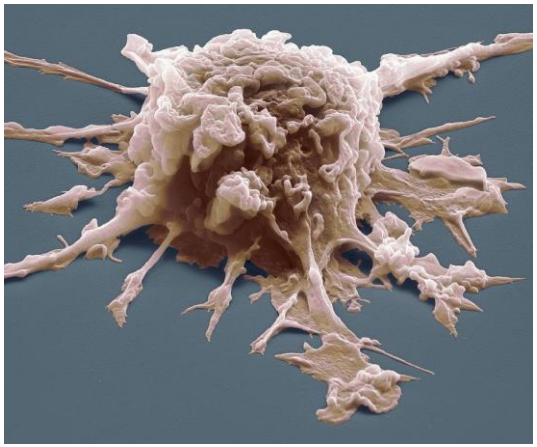


role of neutrophils

- **phagocytosis and secretion of proteases** (elastase, cathepsin G, proteinase 3..) **and antimicrobial peptides** (cationic peptides) kills local bacteria, degrade necrotic tissue and act as chemoattractants for other cells that are involved in the inflammatory phase.

- Neutrophils release mediators (TNF-α, IL-1β and IL-6), which amplify the inflammatory response and stimulate VEGF and IL-8 for repair response.

Role of macrophages



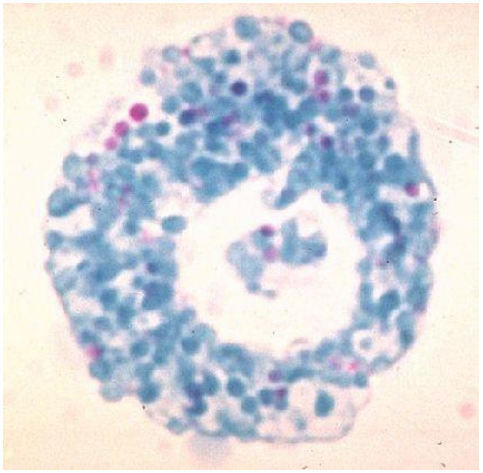
- about 3 days after injury macrophages enter the zone of injury and support the ongoing process by performing **phagocytosis of pathogens and cell debris** and by the **secretion of growth factors, chemokines and cytokines**.

- The inflammatory response to injury is essential for supplying growth factor and cytokine signals that are responsible for cell and tissue movements, which are crucial for the subsequent repair mechanisms in adult mammalians .

- S**ynthesis of numerous potent growth factors** such as TGF- α, TGF-β, basic FGF, PDGF and VEGF, which promote cell proliferation and the synthesis of extracellular matrix (ECM) molecules by resident skin cells

Inflammation

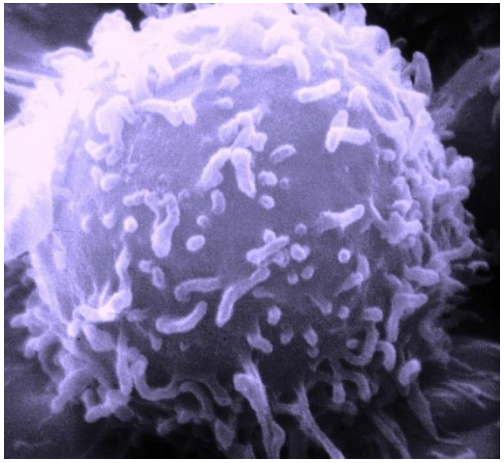
Role of mast cells



- Main role – **release of histamine**. Histamine causes the dilatation of arterias and increases permeability of venules and serves to prevent coagulation of the excess tissue fluid and blood components

- In addition to histamine, mast cell granules contain **serotonin and heparin**, which lead in part to the initial short-lived **increase in permeability of venules**.

Role of lymphocytes



- Lymphocytes - the last cells to enter the wound site in the late inflammatory phase
- attracted 72 h after injury by the action of interleukin-1 (IL-1), complement components and immunoglobulin G (IgG) breakdown products.
- The IL-1 plays an important role in **collagenase regulation**, which is later needed for **collagen remodelling**, **production of extracellular matrix** components and their **degradation**.

Proliferation

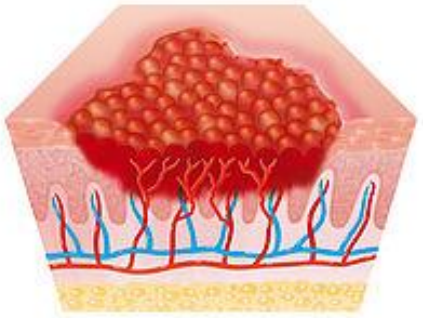
While haemostasis and inflammation were crucial for stabilization of situation in wound, the proliferation means „construction“ of new tissue. Granulation phase involve 3 simultaneously running processes:

1. **granulation** - fibroblast proliferation, migration into wound fibrin clot, and production of new collagen and other matrix proteins, which contribute to the formation of granulation tissue.

2. **angiogenesis** - new vessel growth (mainly by the sprouting of preexisting vessels adjacent to the wound).

3. **reepithelization** - restoring an intact epidermis

Proliferation



Granulation

fibroblasts in the wound edges begin to proliferate and by approximately day 4 start to migrate into the provisional matrix of the wound clot.
The granulation tissue is characterized by a high density of fibroblasts, granulocytes, macrophages, capillaries and loosely organized collagen bundles.

The dominating cell in this phase is the **fibroblast**, which fulfils different functions such as the **production of collagen and ECM substances** (i.e. fibronectin, glycosaminoglycans and proteoglycans).

Granulation tissue has a typical appearance – red (due to rich vascularization), granulous (newly synthesized matrix has low degree of architecture).

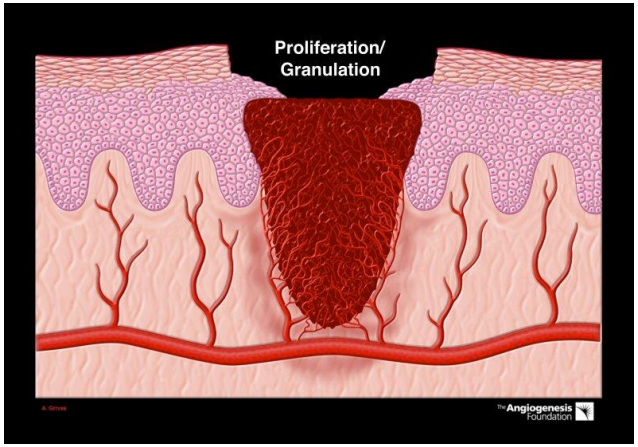
Formation of the ECM - provides a scaffold for cell adhesion and critically regulates and organizes the growth, movement and differentiation of the cells within it.

Proliferation

Angiogenesis

The restoration of the vascular system of the skin is a complex cascade of cellular, humoral and molecular events in the wound bed to reconnect to the nutritive perfusion.

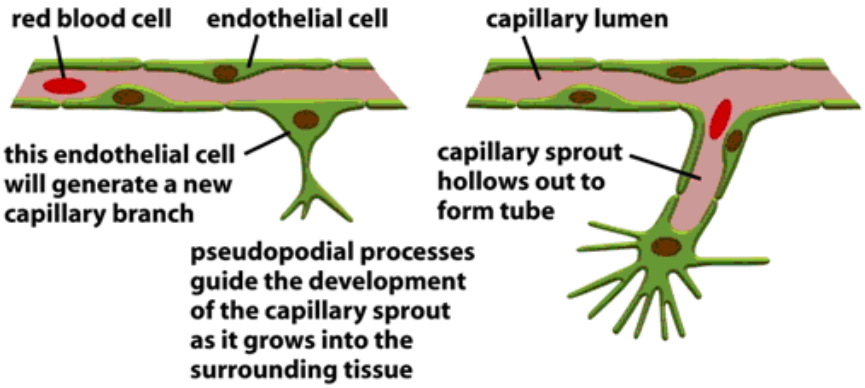
Angiogenesis is triggered by growth factors, e.g. VEGF, PDGF, bFGF and the serine protease thrombin.



Promoting proliferation and growth of endothelial cells is stimulated by hypoxic condition!

Proliferation

Angiogenesis



4-step process:

1. growth factors bind to receptors on the signaling cascades

2. activated endothelial cells secrete proteolytic enzymes dissolving the basal lamina. The endothelial cells are now able to proliferate and migrate into the wound - "sprouting". Orientation of endothelial cells is enabled by adhesion molecules (e.g. integrins).

3. At the front of proliferation the endothelial cells release matrix metaloproteinases lysing the surrounding tissue for the endothelilal proliferation.

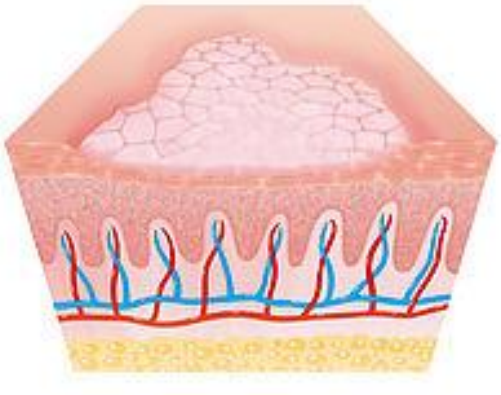
4. differentiation of new vessels into arteries and venules

At the beginning the vessels are arranged radially (*sola cutis se reficientis*). With the wound closure, the inner vessel ring disappears and a new dermal vascular network is formed.

Proliferation

Reepithelization

- Migration of epithelial cells - **keratinocytes** starts from the wound edges within a few hours of wounding.
- A single layer of cells initially forms over the defect, accompanied by a marked increase in epithelial cell mitotic activity around the wound edges.
- Cells migrating across them attach to the provisional matrix below.
- When the advancing epithelial cells meet, migration stops and the basement membrane starts to form.



Maturation

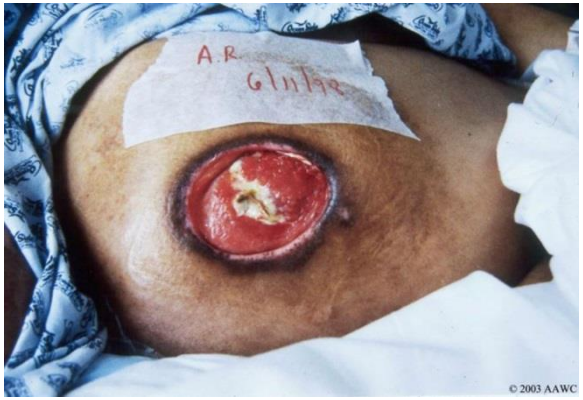
- development of new epithelium and final scar tissue formation

- collagen bundles increase in diameter and hyaluronic acid and fibronectin are degraded. The tensile strength of the wound increases progressively in parallel with collagen collection;
- the initial deposition of collagen bundles is highly disorganized, the **new collagen matrix becomes more oriented and cross-linked over time**. Its subsequent organization is achieved during the final stages of the remodeling phase, to a greater extent by the wound contraction that has already begun in the proliferative phase;
- the underlying connective tissue shrinks in size and brings the wound margins closer together, owing to fibroblast interactions with the extracellular matrix. The process is regulated by a number of factors, with PDGF, TGF-β and FGF being the most important;

As the wound heals, the **density of fibroblasts and macrophages is further reduced by apoptosis**. With time, the growth of capillaries stops, blood flow to the area declines and metabolic activity at the wound site decreases. The end result is a fully matured scar with a decreased number of cells and blood vessels and a high tensile strength.

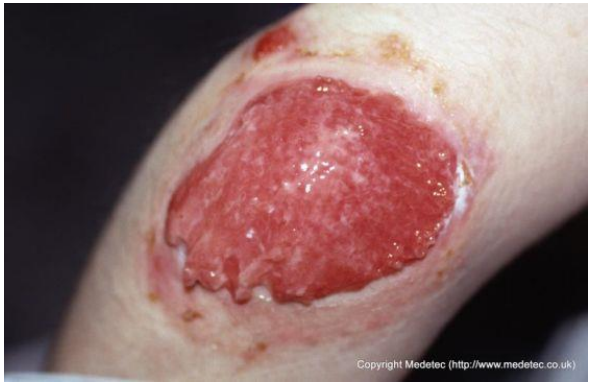
Pathophysiology of granulation

Hypogranulation – the result of the lack of stimulation of the cells. The tissue is formed slowly and in insufficient quantities. The main causes are: diabetes, circulation disorders, infection, senescence, malnutrition, etc. A very common cause is the use of inappropriate wound dressings!



Hypogranulation

Hypergranulation – Granulation tissue is light red or dark pink in colour, with a profusion of often friable capillary loops. This results in an overgrowth of clustered, cobblestoned, bleeding material with a red, raised, glistening surface. It is moist and soft to the touch. It is usually raised from the base of the wound bed.



Hypergranulation

Main causes of hypergranulation:
- prolonged physical irritation or friction with continued repetitive minor trauma or pressure
- excessive inflammation, bacterial bioburden (critically colonised wounds),
- negative pressure suction with microdeformation particularly applicable to large pore foam dressings

Additionally, low oxygen levels and high moisture can stimulate granulation tissue formation!

Pathophysiology of scaring

In adult humans all full thickness cutaneous wounds result in scar. If similarity of the newly formed tissue is >80% of the original wounded skin, the healing proces was normal. Typically, scar does not contain hair folicles or sweat glands.

3 main types of scars:

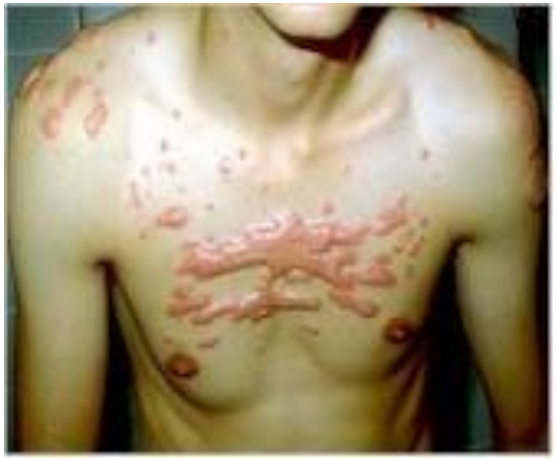
1) **hypertrophic** – **overexpressed synthesis of collagen-** deposits of excessive amounts of collagen which gives rise to a raised scar.

2) **keloid** - firm, rubbery lesions or shiny, fibrous nodules, which can vary from pink to the colour of the patient's flesh or red to dark brown in color. Formation of a type of scar which, depending on its maturity, is composed mainly of either type III (early) or type I (late) collagen. **It is a result of an overgrowth of granulation tissue** (collagen type III) at the site of a healed skin injury which is then slowly replaced by collagen type I.

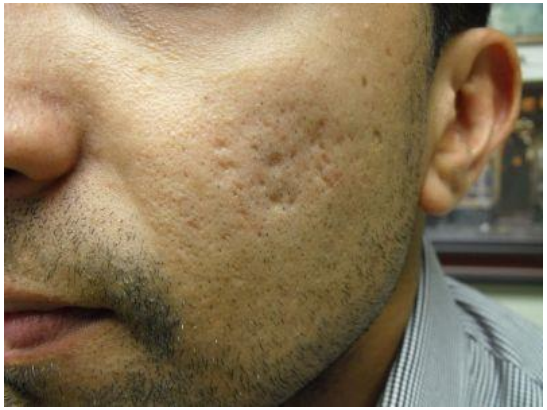
3) **hypotrophic** – mostly a **result of healing of infected wounds**: pathogens (*Staphylococci, Streptococci, Propionibacterium acnes*..) produces enzymes degrading extracelullar matrix in dermis (proteases, hyaluronate-lyases…). The dermis looses its original architecture.

Pathophysiology of scaring

hypertrophic scars



keloid scars



hypotrophic scars

Pathophysiology of scaring

The main known causes of excessive scarring are:

- **TGFβ1 and TGFβ2** - a key factors in the proliferative phase of wound healing; promote signaling via SMAD and Wnt–dependent pathways to enhance scarring (in contrast, **TGFβ3** is receptor antagonist and thus reduces scarring);

- **IL-6 and IL-8** - Proinflammatory cytokines expressed immediately after cutaneous injury; recruit and activate inflammatory cells, thereby promoting scarring (**IL-10,** antiinflammatory cytokine that reduces scarring; it inhibits the infiltration of neutrophils and macrophages towards the wound site and dampens the expression of proinflammatory cytokines);

- **Homeobox13** - transcription factor; absent from the scar-free healing wounds of foetuses; favors fibroplasia;

- **Wnt signaling pathway** - aberrant transduction has been implicated as causative factor of aggressive fibromatosis; hypertrophic scars and keloids display excessive signaling via the Wnt pathway;

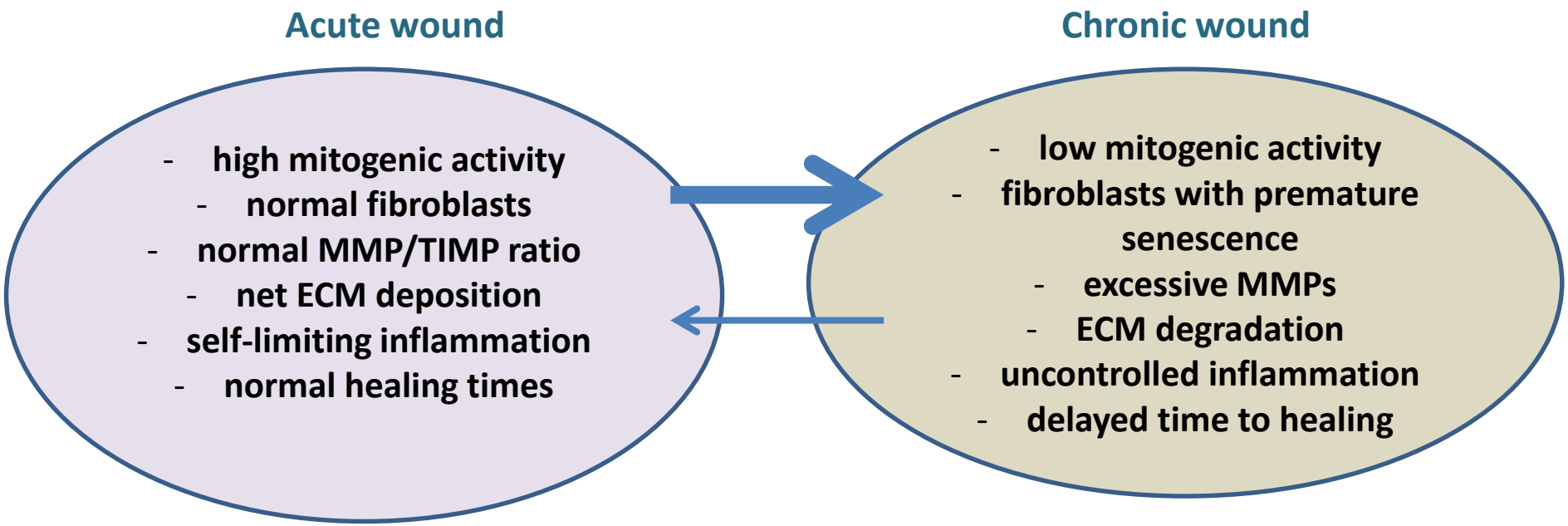
- **PDGF** - secreted by macrophages during the proliferative phase of wound healing and induces fibroblasts to produce type III collagen and exocytose osteopontin; overexpressed in hypertrophic scars and keloids;

- **Osteopontin** - extracellular glycoprotein that enhances fibroplasia; connects integrins on cell surfaces to collagen within the extracellular matrix and promotes cell adhesion and cellular migration; abolition of osteopontin reduces the trafficking of both inflammatory cells and fibroblasts and also leads to a larger number of these cells dying by apoptosis.

Pathophysiology of the healing of chronic wounds

Chronic wound - a wound that fail to proceed through the normal phases of wound healing in an orderly and timely manner.

Most of chronic wounds remain in the inflammatory stage!



Pathophysiology of the healing of chronic wounds

- The problem with the excess of MMP activity in chronic wounds can be smartly solved by the use of collagen – based dressings which serve as an alternative substrate for these enzymes.

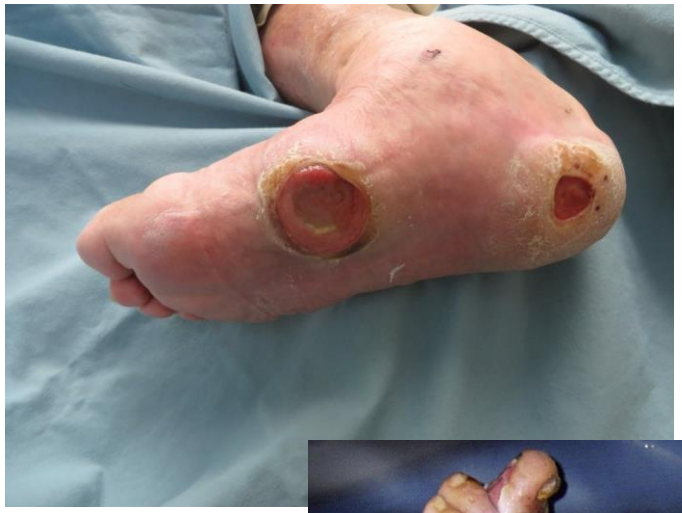
The foam wound dressing made of atelo-collagen:

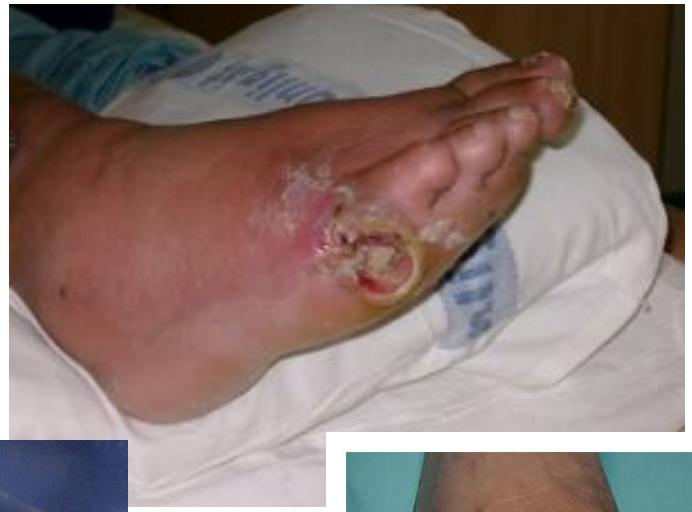
Pathophysiology of the healing of chronic wounds

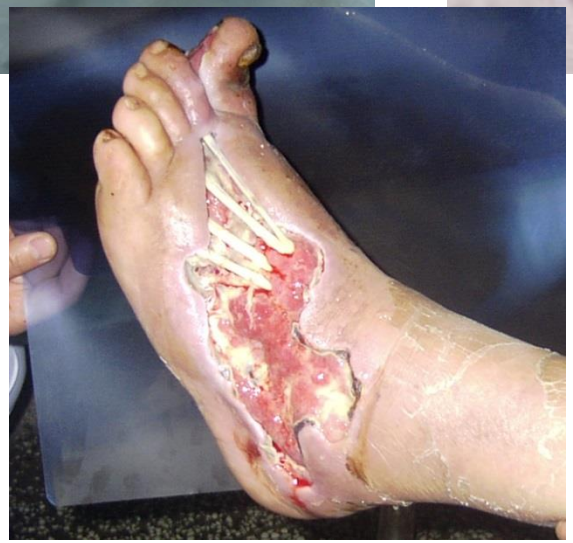
Diabetic wounds
Pressure ulcers
Leg ulcers
Infected wounds

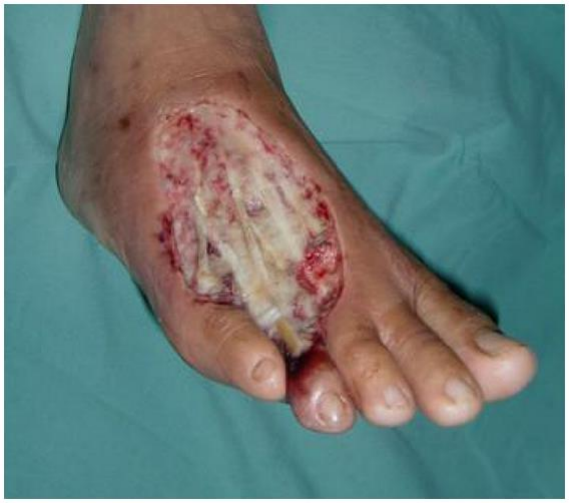
Pathophysiology of diabetic wounds

The most common type of diabetic wound is the foot ulcer:









Pathophysiology of diabetic wounds

Pathogenesis of foot ulceration

Two major underlying causes:

1) peripheral neuropathy
2) ischemia from peripheral vascular disease

Pathophysiology of diabetic wounds

Role of neuropathy

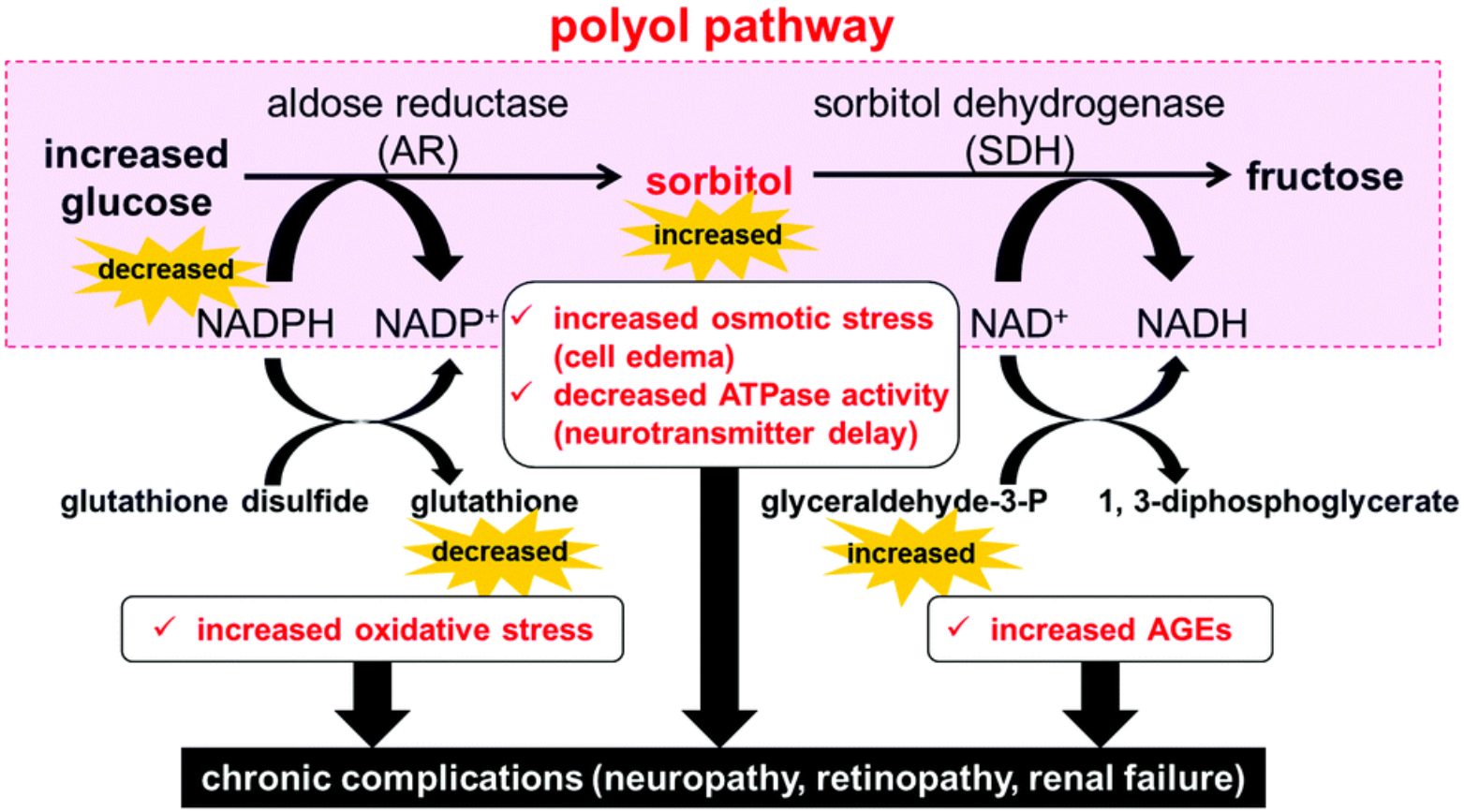
- a cause of more than 60% of diabetic foot ulcers
- a result of **hyperglycemia-induced metabolic abnormalities** – especially in the polyol pathway:

  Hyperglycemia increases activity of **aldose reductase** and **sorbitol dehydrogenase**. This results in the conversion of intracellular glucose to **sorbitol** and **fructose** in neurons.

  The accumulation of sorbitol and fructose in cells lead to decreased synthesis of **myoinositol** which is essential for normal interneuronal conduction.

  Conversion of glucose to sorbitol ad fructose results in a depletion of NADP stores which are necessary for detoxification of ROS and for synthesis of vasodilatator nitric oxide => increase in **oxidative stress in neurons**, increase in vasoconstriction and ischemia => **neuron injury and death**.

Pathophysiology of diabetic wounds

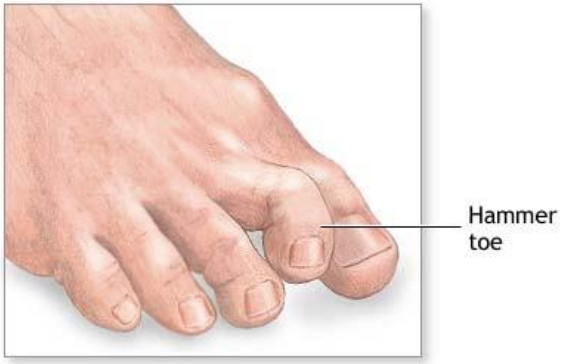


Pathophysiology of diabetic wounds

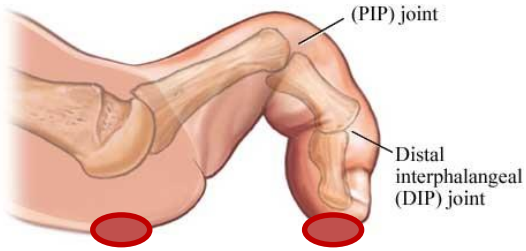
Role of neuropathy

Peripheral neuropathy leades to the **loss of sensation** in lower extremities

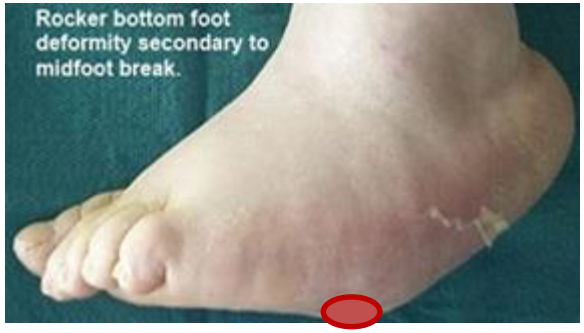
- Patients are often unable to detect the insult => many wounds go unnoticed and progressively worsen as the site is continuously subjected to traumatization.
- Imbalance in the innervations of the foot muscles from neuropathic damage can lead to the development of **deformities** (hammer toe, claw toe, the Charcot arthropathy):



hammer toe



claw toe



The Charcot arthropathy

Pathophysiology of diabetic wounds

Role of vascular disease

- a cause of more than 50% of diabetic foot ulcers

a result of hyperglycemia-induced metabolic abnormalities in endothelilal cells and smooth muscle cells in peripheral arteries:

- Lack of endothelium-derived vasodilators => **constriction**

- Increase in tromboxane A2, a vasoconstrictor and platelet aggregation agonist => increased risk for **plasma hypercoagulability**

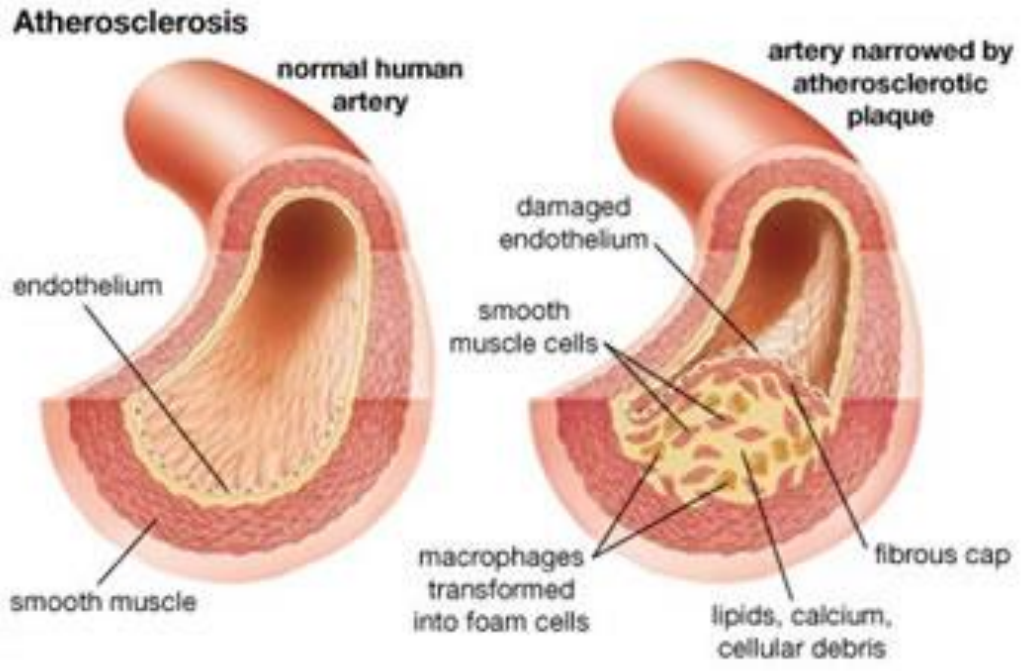
- Alterations in the vascular extracellular matrix => **stenosis of the arterial lumen**

Pathophysiology of diabetic wounds

Role of vascular disease

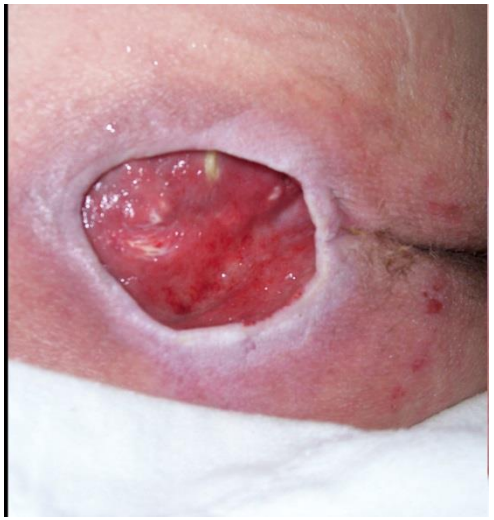
Peripheral arterial disease is complicated by other factors:

- Smoking
- Hypertension
- Hyperlipidaemia



Pathophysiology of the pressure ulcers

pressure ulcers — are injuries to skin and underlying tissue resulting from **prolonged pressure** on the skin. PU most often develop on skin that covers bony areas of the body, such as the heels, ankles, hips and tailbone.



pressure ulcer - decubitus

People most at risk of PU are those with a medical condition that limits their ability to change positions, requires them to use a wheelchair or confines them to a bed for a long time.

The main pathophysiological mechanism is **ischemization** of the compressed area:

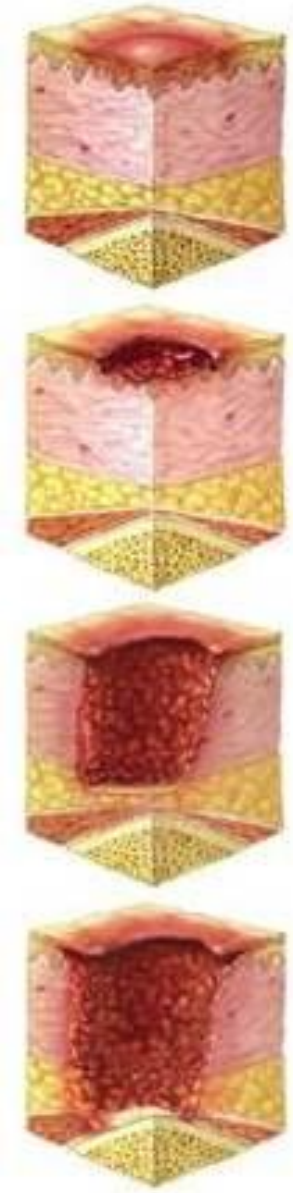
- **increasing capillary permeability**, particularly after pressure is released
- **increasing interstitial oedema**
- **blocking lymphatic and venous drainage**
- **occluding vessels** causing hypoxia, ischaemia and tissue necrosis.

PU can develop quickly and are often difficult to treat.

The most common type of PU is the **bedsore** (decubitus)

Pathophysiology of the pressure ulcers

For the purposes of and treatment, it is helpful to stage the pressure ulcer according to the system promulgated by the **National Pressure Ulcer Advisory Panel** (NPUAP), as follows:



- (Suspected) deep tissue injury - A purple or maroon localized area of discolored intact skin or blood-filled blister due to damage of underlying soft tissue from pressure or shear
- Stage I - Intact skin with signs of impending ulceration, initially presenting erythema indicating reactive hyperemia
- Stage II - A partial-thickness loss of skin involving epidermis and dermis
- Stage III - A full-thickness loss of skin with extension into subcutaneous tissue but not through the underlying fascia
- Stage IV - A full-thickness tissue loss with extension into muscle, bone, tendon, or joint capsule
- Unstageable - A full-thickness tissue loss in which the base of the ulcer is covered by slough or eschar to such an extent that the full depth of the wound cannot be determined

Pathophysiology of the leg ulcers

The etiology of vascular ulcers may be:

1. venous
2. arterial

… or both

Venous Ulcer

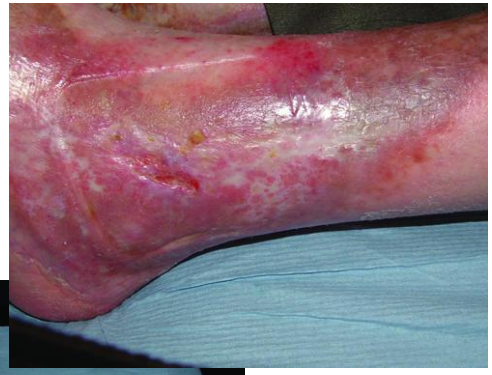
- Found on the medial side of the ankle, above the ankle bone
- Shallow and superficial
- Can be small or large, but the edges are NOT A PERFECT CIRCLE
- Almost always there will be edema (swelling of the leg) and in fact, edema is usually the FIRST thing you will find
- Wet all the time
- May have an odor
- Usually there is no pain with walking, per se, but the patient will say it hurts to stand up. Once they are up and start walking, the pain subsides

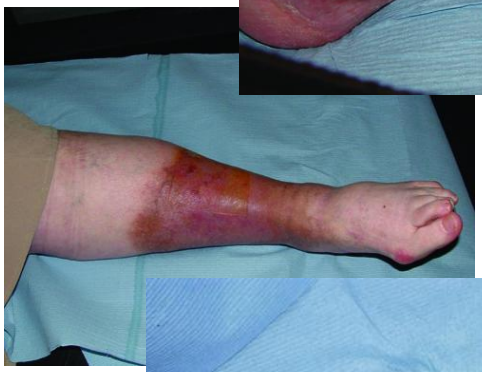
Arterial Ulcer

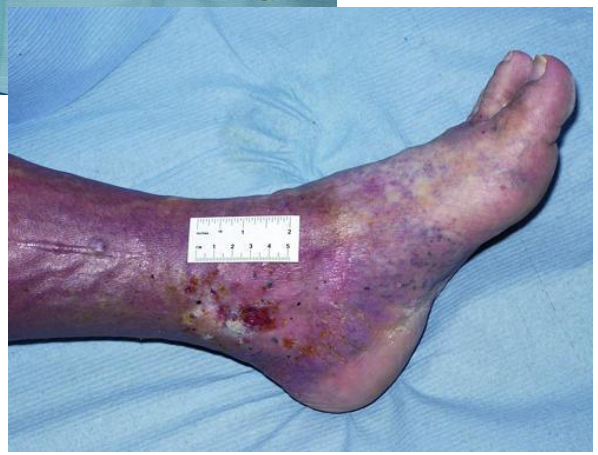
- May be found on tips of toes, between the toes or on the lateral side of ankles
- Most likely are perfectly round, smooth edges
- May or may not have swelling (edema) of the lower extremities
- Lower extremity may be cool to touch, skin is pale shine, taut, and thin
- Minimal drainage
- No odor
- Skin on lower extremities often tight, hard, shiny
- Skin is often cool or cold to touch
- There may not be any hair on the toes or on the legs
- There may be pain with walking (claudication)
- Faint to absent pedal (foot) pulse

Pathophysiology of the leg ulcers

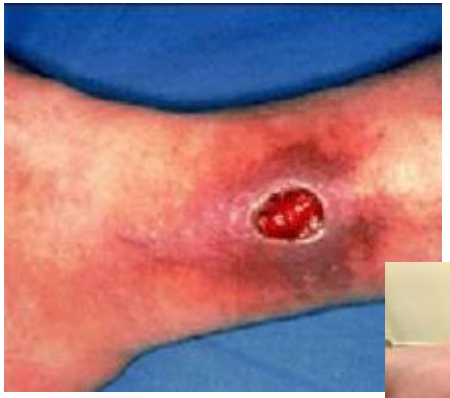
venous ulcerations

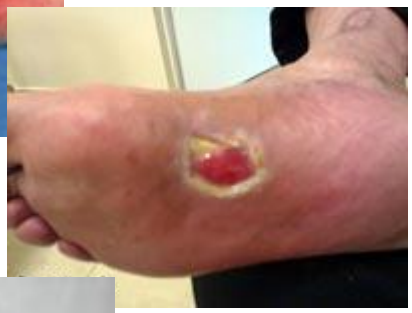


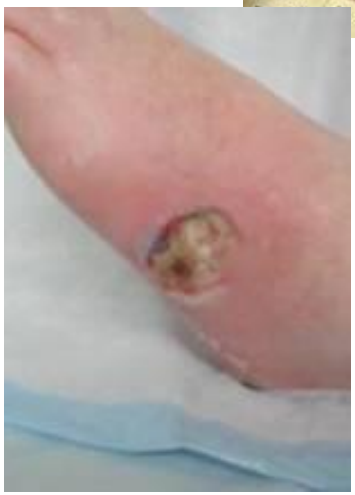




arterial ulcerations







Pathophysiology of the venous leg ulcers

Venous leg ulcers (VLUs) - open lesions between the knee and ankle joint that occur in the presence of venous disease. They are the most common cause of leg ulcers, accounting for 60-80% of them. Over the age of 65, the prevalence increases to 4%. On an average 33-60% of these ulcers persist for more than 6 weeks and are therefore referred to as chronic VLUs.

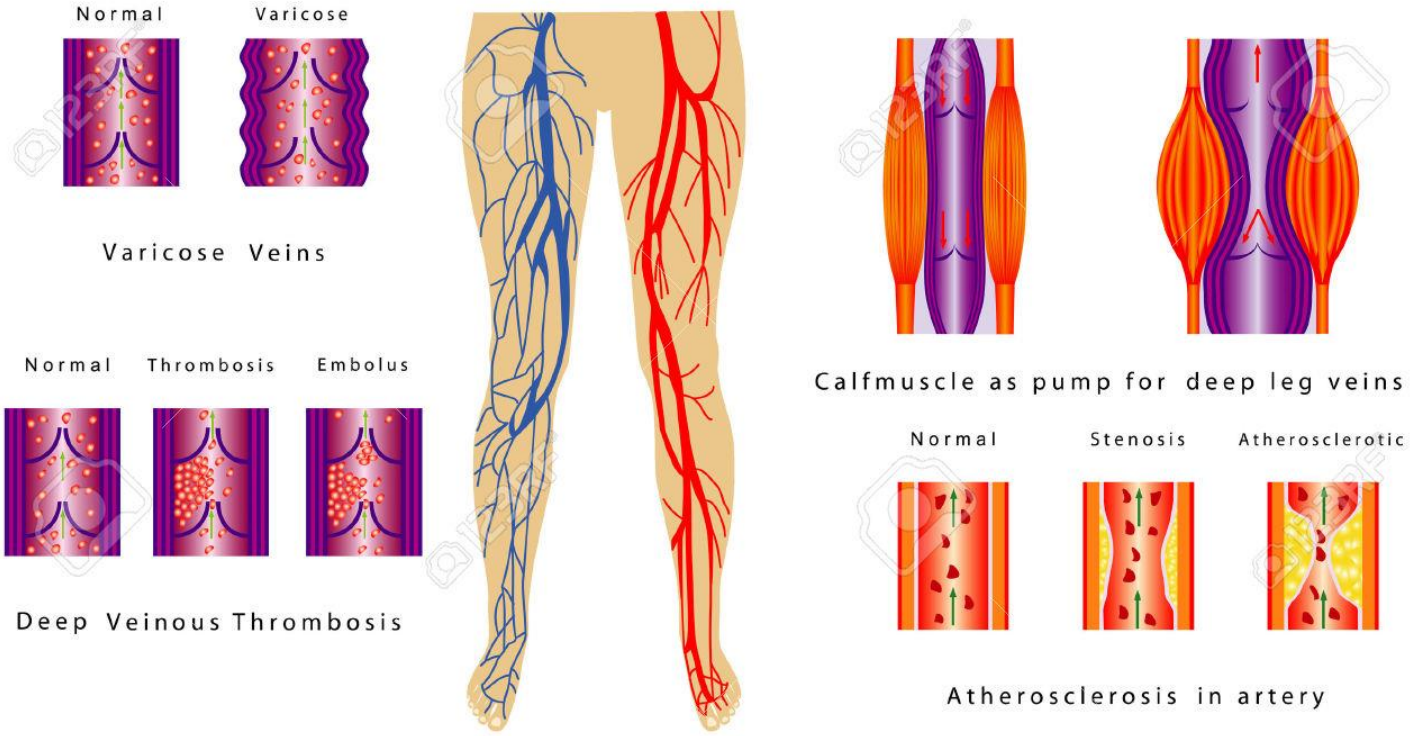
The most important pathophysiological mechanisms are:

- Venous hypertension
- Increased fibrin stores in perivascular space (fibrin cuff theory)
- Inflammation (inflammatory trap theory)
- Dysregulation of cytokines

Pathophysiology of the venous leg ulcers

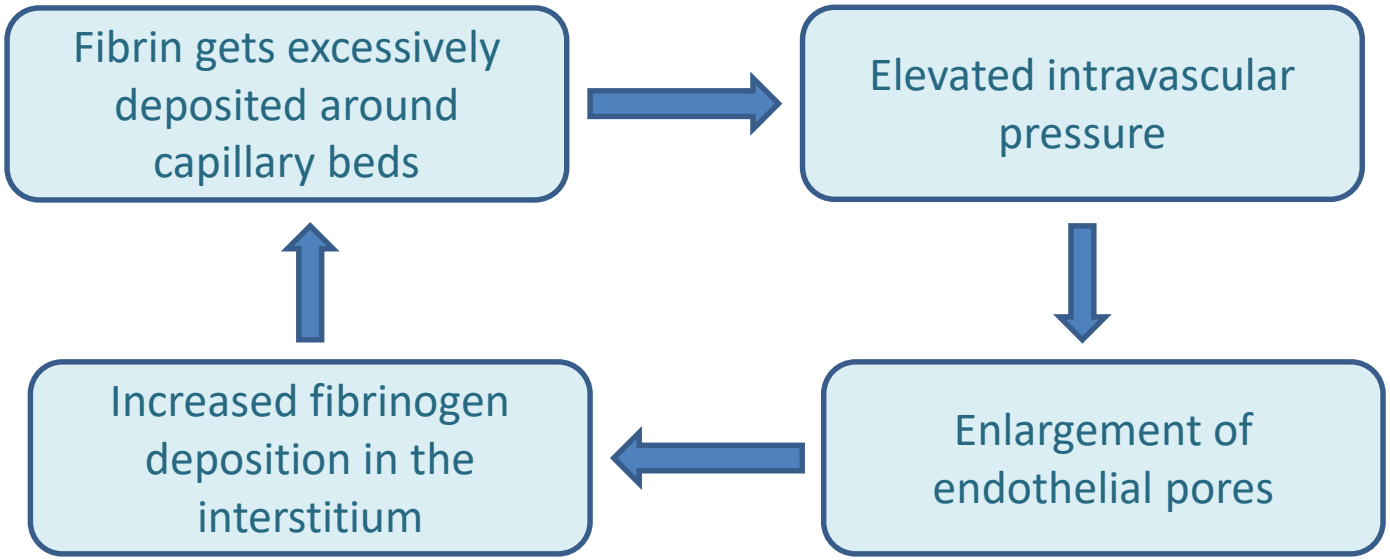
Venous hypertension

Deep vein thrombosis, perforator insufficiency, superficial and deep vein insufficiencies, arteriovenous fistulas and calf muscle pump insufficiencies lead to **increased pressure in the distal veins** of the leg and finally to **venous hypertension**.



Pathophysiology of the venous leg ulcers

Increased fibrin stores in perivascular space (fibrin cuff theory)



The “fibrin cuff” which surrounds the capillaries in the dermis may decrease oxygen permeability 20-fold => hypoxia, impaired healing

Pathophysiology of the venous leg ulcers

Inflammatory trap theory

Various growth factors and inflammatory cells, which get trapped in the fibrin cuff promote **severe uncontrolled inflammation** in surrounding tissue preventing proper regeneration of wounds.

Leukocytes get trapped in capillaries, releasing **proteolytic enzymes** and **reactive oxygen metabolites** => endothelial damage.

These injured capillaries become increasingly **permeable to various macromolecules**, accentuating fibrin deposition.

Occlusion by leukocytes also causes local ischemia thereby increasing tissue hypoxia and reperfusion damage.

Pathophysiology of the venous leg ulcers

Dysregulation of cytokines

Dysregulation of various pro-inflammatory cytokines and growth factors like TNF-α, TGF-β and matrix metalloproteinases lead to chronicity of the ulcers.

Other known factors

Thrombophilic conditions like factor V Leiden mutation, prothrombin mutations, deficiency of antithrombin, presence of antiphospholipid antibodies, protein C and S deficiencies and hyperhomocysteinemia are also implicated.

Pathophysiology of the arterial leg ulcers

Arterial ulceration is due to a **reduced arterial blood supply to the lower limb**. The most common cause is atherosclerotic disease of the medium and large sized arteries.

Further damage to the arterial system occurs with concurrent hypertension through damage of the intimal layer of the artery.

The reduction in arterial blood supply results in tissue hypoxia and tissue damage => necrosis => ulceration.

Thrombotic and atheroembolic episodes may contribute to tissue damage and ulcer formation

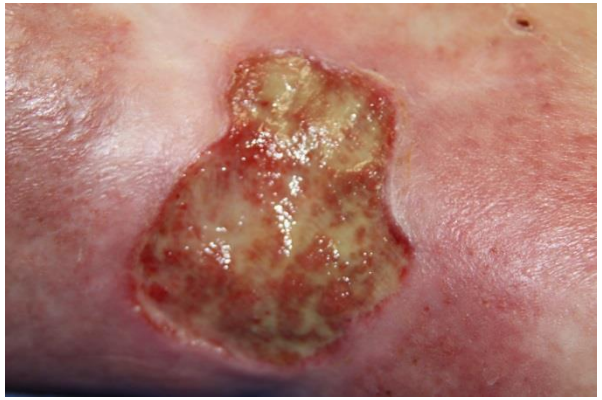
Other causes include diabetes, thromboangiitis, vasculitis, pyoderma gangrenosum, thalassemia, and sickle cell disease, some of which may predispose to the formation of atheroma.

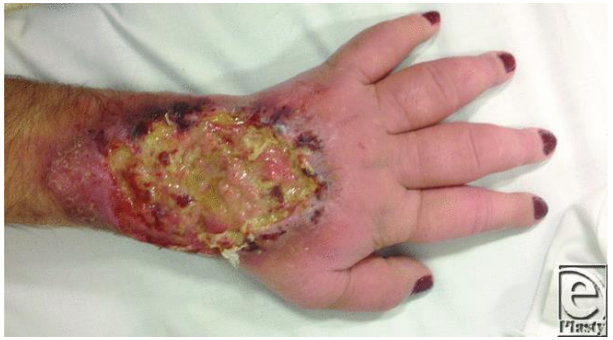
Pathophysiology of the infected wounds

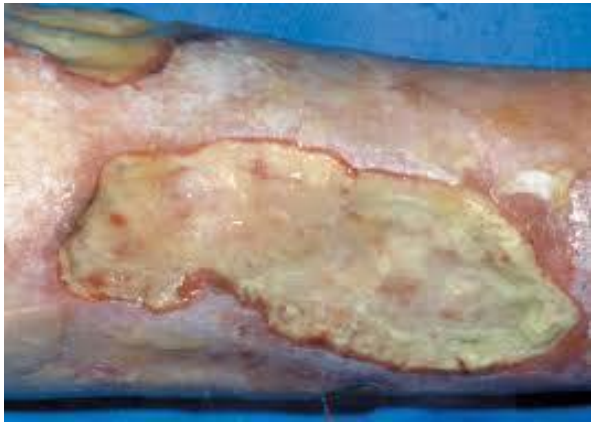
Most wounds contain micro-organisms and many heal successfully.
However, sometimes micro-organisms (particularly bacteria) multiply, invading and damaging tissues, delaying healing and occasionally causing systemic illness.

The potential for bacteria to produce harmful effects is influenced by the:

- **ability of the patient's immune system** to combat the bacteria (host resistance)
- **number of bacteria introduced** – higher numbers are more likely to overcome host resistance
- **type of bacteria introduced**:
  - some bacteria have greater disease-producing ability (virulence) than others, and may be able to cause disease in relatively low numbers
  - benign residents in one body site may cause disease if transferred elsewhere.







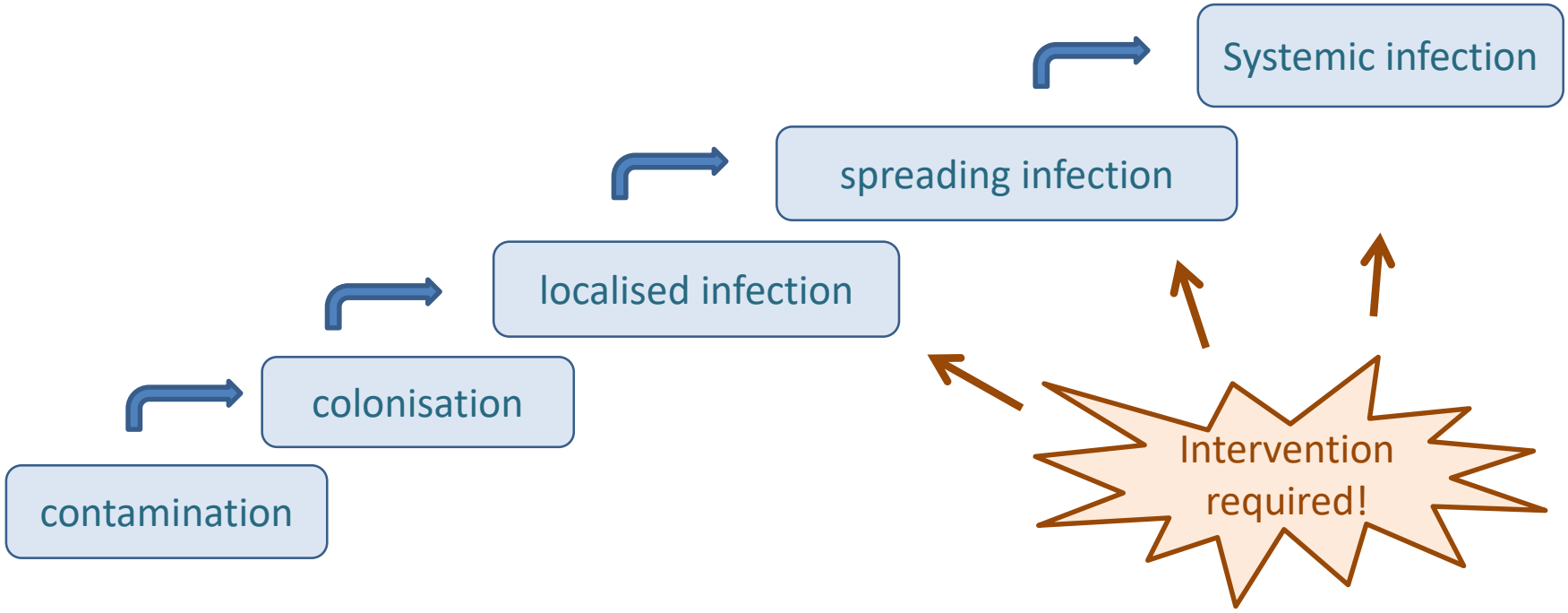
Pathophysiology of the infected wounds

The presence of bacteria in a wound may result in:

contamination – the bacteria do not increase in number or cause clinical problems

colonisation - the bacteria multiply, but wound tissues are not damaged

infection– the bacteria multiply, healing is disrupted and wound tissues are damaged (local infection). Bacteria may produce problems nearby (spreading infection) or cause systemic illness (systemic infection).



Pathophysiology of the infected wounds

The most common wound pathogens are aerobic or facultative pathogens such as:
Staphylococcus aureus,
Pseudomonas aeruginosa,
beta-hemolytic streptococci

Other wound bacteria are:

Staphylococcus epidermidis ,
Escherichia coli,
Enterococcus faecalis …

Biofilm

- a consortia of bacteria (but also fungi or virues) embedded in a self-secreted and/or host-derived, self-hydrated polymer matrix (EPS). The matrix provides an optimal environment for microbial cells survival, enabling their escape from immune system and resistance to antibiotic treatment, and giving a structure in which the bacteria can grow and communicate with each other.

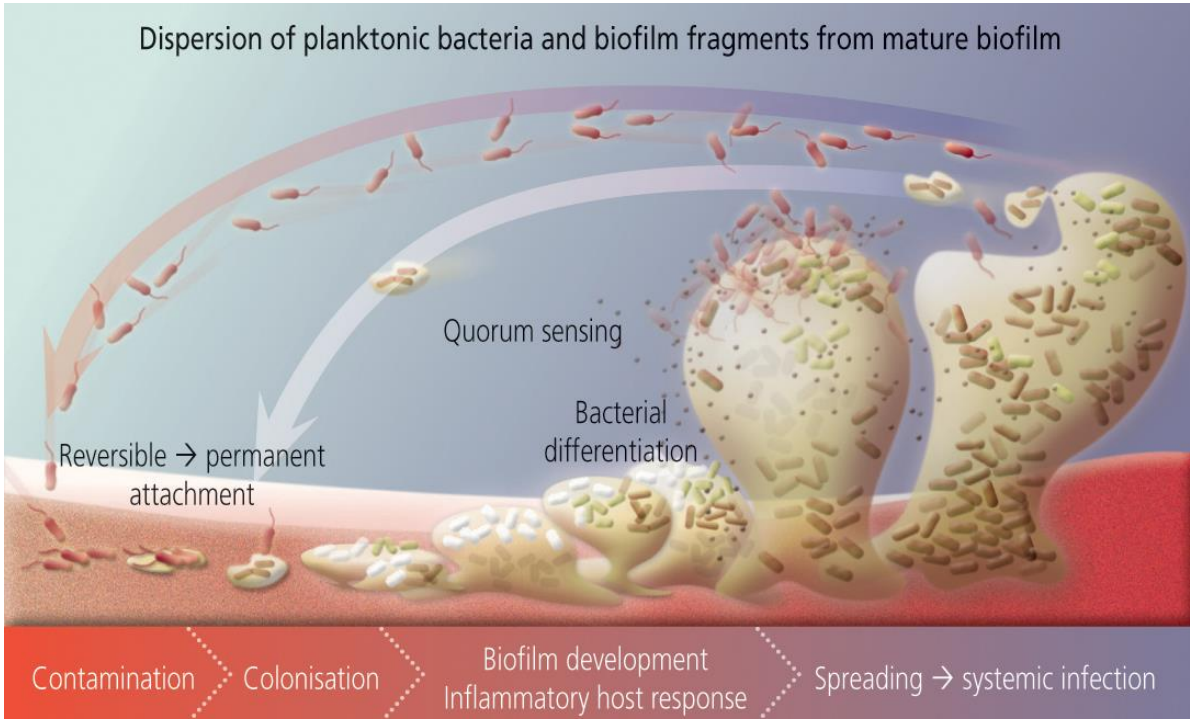
Pathophysiology of the infected wounds

Biofilm

Bacterial biofilms develop as a natural part of how bacteria grow outside of the laboratory (in vivo).

There are several key stages in biofilm development:

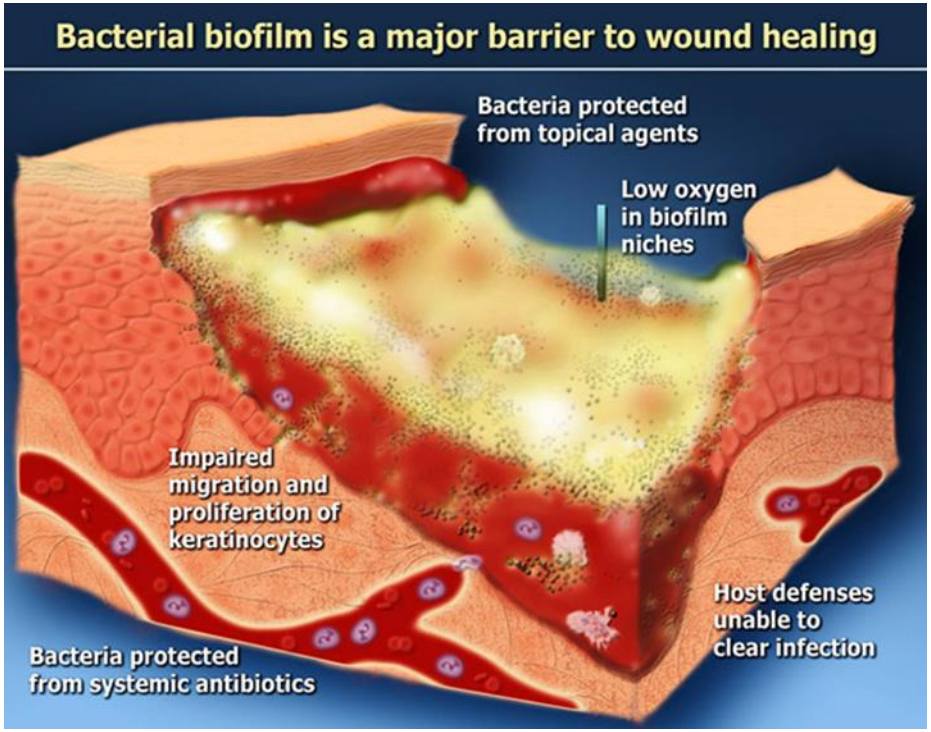
- Bacteria attach to the surface or each other, first reversibly and then irreversibly
- Bacteria produce a substance (extra-polymeric substance) in which they live
- Bacteria multiply and continue to produce more substances, which play an important role in bacteria communicating with each other



Small amounts of bacteria are shed from the mature biofilm when there are not enough nutrients to support them. These bacteria can go on to form biofilms elsewhere.

Pathophysiology of the infected wounds

In infected wounds - a **significantly lower diversity** of bacteria => normal skin microbiota protects skin against colonization, proliferation and dissemination of opportunistic and pathogenic microorganisms



Heavily infected wound persits in the **inflammatory** stage. Moreover, live bacteria and their toxins induce excessive inflammatory response and tissue damage that can lead to abscesses, cellulitis, osteomyelitis or limb loss. The most of bacterial wound pathogens product several enzymes causing **destruction of connective tissues** (proteases, hyaluronate-lyase) and immune cells (leucocidins).

Pathophysiology of the infected wounds

Therapeutical strategies of the heavily infected wounds are mainly based on eradication of biofilm:

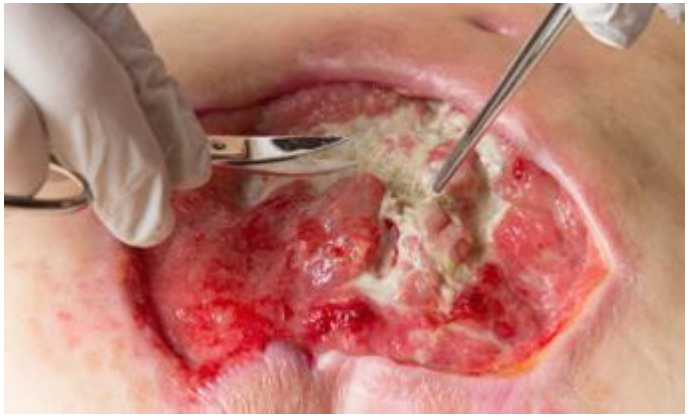
Antimicrobial therapy

- local antibiotics are almost uneffective against biofilm!
- Necessary to use antiseptics (the most effective is iodine, superoxidizied solutions, octenidine, silver, chlorhexidine..)

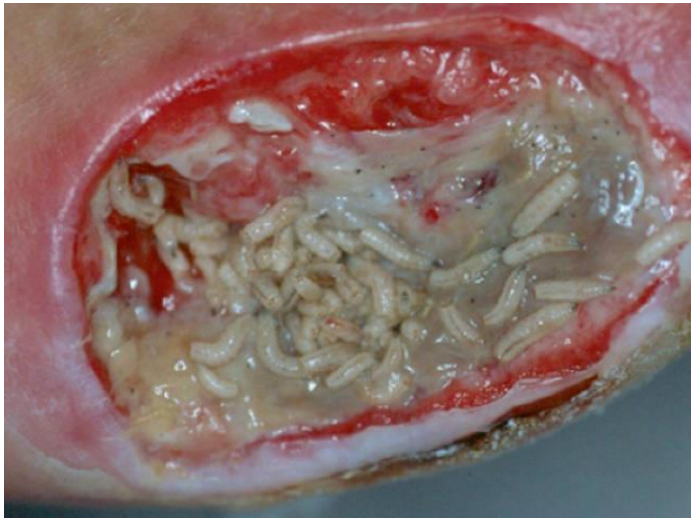
Débridement - removal of biofilm and contaminated tissue

- Surgical (sharp débridement using forceps or other sharp surgical instruments)
- Enzymatic – enzymatic lysis of the contamined tissue
- Maggots – very effective, minimizes traumatization and pain
- Ultrasound

Appropriate wound dressings which could prevent restoration of biofilm.



Surgical (sharp) débridement



Débridement using maggots

If necessary, you can contact me at:

Pavel Klein, Ph.D.
Animal Research and Care Facility
Institute of Pathological Physiology

E-mail: pavel.klein@lfp.cuni.cz

Phone: +420 775 224 887