The Art of Balancing the wound micro-environment

HOW TO TREAT CHRONIC WOUNDS IN ANOTHER WAY

Dermagenics BV
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1. WOUND HEALING PROCESS

The wound healing process is initiated by almost any type of tissue damage.
The process is a complex succession of overlapping events involving a variety of different cell populations.

The four overlapping stages are:

1. Hemostasis
2. Inflammatory stage
3. Cell proliferation and matrix repair stage
4. Remodeling stage

1.1. HEMOSTASIS

Disruption of the skin layers and vasculature leads to the entry of platelets into the extra cellular matrix where they degranulate. This degranulation results in the initiation of hemostasis and the recruitment of additional cell types to the area of injury. The platelets initiate the healing process by releasing a number of soluble mediators, including growth factors. These rapidly diffuse from the wound and inflammatory cells are drawn to the area of the injury.

1.2. INFLAMMATION

The inflammatory phase is initiated by the blood clotting and platelet degranulation process.
During this phase there is significant vasodilation, increased capillary permeability, complement activation, and migration of polymorphonuclear leukocytes (PMN) and macrophages to the site of the wound.
The neutrophils and macrophages engulf and destroy bacteria and release proteases, including elastase and collagenase, which degrade damaged ECM components. They also secrete additional growth factors.

Intercellular communication
Communication between cells in the wound bed is achieved not with words and drawings and cell phones, but with chemicals. The chemicals that cells use to communicate with each other include various soluble mediator proteins (e.g. growth factors and cytokines).

Growth Factors:
Growth Factors deliver messages to non-immune cells which results in cell proliferation or synthesis of granulation tissue.
A particular type of cell may produce many types of growth factors or several different types of cells may all produce a particular growth factor.

Cytokines:
Inflammation is largely regulated by a class of molecules called cytokines, which have powerful stimulatory and inhibitory actions on inflammatory cells.
Cytokines are identified according to their influences on chemotaxis, proliferation and differentiation of inflammatory cells.
Cytokines have also important actions on wound cells.
They stimulate production of proteases by fibroblasts.
Macrophages attract further macrophages and continue to stimulate migration of fibroblasts, epithelial cells and vascular endothelial cells into the wound to form granulation tissue around 5 days after injury.

Growth factors and cytokines can be considered the ***"words"*** of the chemical language of cells. For the transmission of the message, it must travel through the wound environment and then bind to a specific area on the surface of another cell.
These binding sites are called cell surface receptors and can be considered as ***"ears".***

Chemokines:
A third important group of regulatory proteins that influence wound healing are chemokines. Chemokines have two primary functions:
To regulate the trafficking of leucocyte population during normal health and development
To direct the recruitment and activation of neutrophils, lymphocytes, macrophages, eosinophils and basophils during inflammation.

1.3. CELL PROLIFERATION AND REPAIR OF THE MATRIX

As the number of the inflammatory cells in the wound decreases, the fibroblasts, endothelial cells and keratinocytes take over synthesis of growth factors.
These growth factors continue to promote cell migration, proliferation, new capillary formation and synthesis of extra cellular matrix (ECM) components.
ECM is a complex assortment of proteins and polysaccharides, including

- Collagens
- Elastin
- Fibrin
- Fibronectin
- Vitronectin
- Glycosaminoglycans
- Glycoproteins
- Proteoglycans

Initially, the injury defect is filled by a provisional wound matrix consisting predominantly of fibrin and fibronectin.
As fibroblasts are drawn chemotactically into the matrix, they synthesize new collagen, elastin and proteoglycan molecules that form the initial scar and secrete lysyl oxidase, which cross-links collagen of the ECM.
However, before the newly synthesized matrix components can properly integrate with the existing dermal matrix, all damaged proteins in the matrix must be removed.
This is carried out by proteases secreted by neutrophils, macrohages, fibroblasts, epithelial cells and endothelial cells.
Key proteases include collagenases, gelatinases and stromelysins, which are all members of the matrix metalloproteinase (MMP) super family.
Cell proliferation and synthesis of new ECM places a high metabolic demand on the wound cells, which is met by a dramatic increase in vascularity of the injured area.
Epithelial cells proliferate and migrate across the highly_vascularized, new ECM (granulation tissue), and reform the epidermal layer.

1.4. REMODELING OF SCAR TISSUE

Synthesis of new ECM molecules continues for several weeks after initial wound closure, and the scar is often visibly red and raised. Over a period of several months, the appearance of the scar usually improves, becoming less raised and red.
The increased density of fibroblasts and capillaries present in the early phase of healing declines. In the final remodeling phase, tensile strength reaches a maximum as cross-linking of collagen fibrils plateaus.

2. CHARACTERISTICS OF NON-RESPONDING CHRONIC WOUNDS

Chronic or non healing ulcers are characterized by:
- Defective remodeling of the ECM
- Failure to re-epithelialize
- Prolonged inflammation

Wounds will not respond to any treatment as long as there is an imbalance in the bodies own healing processes with a prolonged inflammatory phase.
One of the characteristics of non-responding wounds is imbalance in cell metabolisms:

- Senescent fibroblasts are characterized by high proteases production
- Still activate neutrophils show an ongoing production of oxygen radicals.

Conditions like diabetes, rheumatoid arthritis and cortisone use will also contribute to the imbalance of the micro-environment of chronic wounds.

Other characteristics of chronic wounds are:

1. Over use of antimicrobials [1]
2. Over production of oxygen radicals
3. Over activation of the complement system [2]
4. Insufficient acidification of micro wound environment [3 4]
5. Imbalance of proteases production

When wounds fail to heal, the molecular and cellular environment of a chronic wound bed must be converted into that of an acute, healing wound so that healing can proceed through the natural sequential phases.

Other characteristics of chronic wounds

2.1. Antimicrobials

- Antimicrobials are highly over used [1]
- There is no proof of principle using antimicrobial agents in chronic wounds, except when there is an acute infection [1]

2.2. Oxygen radicals

2.2.1. Defence mechanisms against bacteria

Infection is one of the main causes of an impaired process of wound healing. The primary function of neutrophils is to build a first border of defense against micro-organisms which invade the body via the wound.
Bacteria will be killed by 2 mechanisms:
One is entirely oxygen-dependent and the other will occur entirely without oxygen.

2.2.2. Oxygen-dependent defense mechanisms

When neutrophils are stimulated, the production of oxygen radicals (O_2 -) will be increased. Interaction between two O_2 – molecules results in the oxidation of one of the molecules and reduction of the other leading to the formation of oxygen and hydrogen peroxide (H_2O_2)

$$\mathbf{O_2^- + O_2^- \xrightarrow[SOD]{} H_2O_2 + O_2}$$

This reaction is catalysed by Super Oxide Dismuthase (SOD). Inactivation of SOD results in a diminished defense against wound infections.
The production of hydrogen peroxide also is important for the antibacterial activity of neutrophils.

Production of toxic oxygen species:

- Hydroxyl radicals (-OH)
- Non radical Hypochloric acid (HClO)

Furthermore, a number of other cytotoxic substances can also be formed, such as hypochloric acid and hydroxyl radicals.
Hydroxyl radicals are formed by the reaction between H_2O_2 and O_2 -.
Iron binding protein (lactoferrin) promotes the production of hydroxyl radicals.
Hydroxyl radicals do not kill only bacteria, but also are harmful to neutrophils itself, all other tissue cells and the surrounding wound and extra-cellular matrix.

2.2.3. Non-oxygen-dependent defence mechanism

Neutrophils can also kill bacteria without the help of oxygen radicals.
Neutrophils contain various proteins that can kill both gram-positive and gram-negative bacteria.
The outermost lipopolysaccharide layer of the bacterial cell wall becomes unstable, so bacteria cannot survive.

2.2.4. The role of fibronectin in the elimination of bacteria

Fibronectin is a glycoprotein which circulates in the bloodstream.
Through fibronectin, it is possible for macrophages to eliminate bacteria.
Fibronectin plays a role in:

- Facilitating cell adhesion to the extra-cellular matrix.
- Adhesion of inflammatory cells to the matrix
- Adhesion of epithelial cells to the fibrin matrix that coats the wound surface.

2.3. The Complement System

Group of serum proteins involved in the control of the immune response and inflammation:

- Cascade activated by interaction with the immune system
- Activation of phagocytes and lytic attack on cell membranes

The human complement system is part of the humoral immune system and plays an important role in the bodies defense mechanism. It consists of more than 30 proteins. Next to its beneficial effects, complement may give rise to tissue damage in many situations, including mechanical injury, formation of immune complexes caused by (auto) antibodies, reperfusion damage and contact with exogenous materials such as prostheses or renal dialysis membranes.

Activation leads to formation of high molecular membrane attack complex that causes death of bacteria through lysis.
In addition small split products are generated, which mediate immuno regulatory effects.
Complement factor C3b has a major biological function.
Micro organisms and foreign particles are covered with C3b. This enables phagocytes with receptor C3b to recognize, ingest and destroy these invaders by producing ROS.
C5a is another activating agent for PMNs. C5a is a major chemotactic factor for these phagocytes.
Inhibition of the complement system promotes wound healing by reducing levels of ROS. Achieved by limiting the factors involved in PMN recruitment and activation.

2.4. Insufficient acidification of micro wound environment

Low pH is important in synthesis of neutral complexes facilitating transport of metal ions over the cell membrane

Features and disadvantages of Acetic acid

- Acidification influences the condition for wound healing [4]
- Acetic acid is used to control wound infection
- Pseudomonas specious can be eliminated by acetic acid 5%
- Risk of increase of Staphylococcus Aureus and Proteus species
- Acetic acid 5% can cause discomfort and pain

2.5. IMBALANCES PROTEASES – Matrix Metalloproteinases (MMPs)

There is increasing evidence that MMPs and their tissue inhibitors, TIMPs, play an important role in the complexly orchestrated events that lead to wound healing.
Imbalance of MMPs in the micro environment of the wound has been associated with poor healing conditions leading to chronic wounds.
Several studies of have shown wound fluid containing high levels of MMPs and low levels of TIMPs in chronic ulcers.

WHAT ARE MMPs?

MMPs are a specific group of proteolytic enzymes
These zinc dependent micro-proteins appear in the extra-cellular matrix (ECM) after tissue has been damaged.

MMPs are a family of structurally related, proteins-degrading enzymes that require Ca^{++} ions for structural conformation and Zn^{+} ions in their active site for function.
About 20 different members have been indentified.

MMPs play an important role in many natural physiological processes such as wound healing, embryonic development and menstruation.

MMPs play an important role in both epithelial degradation and regeneration.
Collectively, the MMP family of enzymes is capable of digesting almost all of the components of the ECM..

MMPs play an important role in remodelling extra-cellular matrix (ECM)

Key players in skin MMP production
Multiple cell types synthesize MMPs

- Granulocytes
- Fibroblasts
- Keratinocyt
- Endothelium
- Mast cells
- Macrophages
- Eosinophiles

The members of the MMP-family

- Collagenases
- Gelatinases
- Stromelysines
- Membrane type MMPs

Zn^{+} and Ca^{++} dependent

Collagenases

1. Collagenase 1 (MMP-1)
2. Collagenase 2 (MM-8)
3. Collagenase 3 (MMP-13)
4. Collagenase 4 (MMP18)

Gelatinases

1. Gelatinase A (MMP-2)
2. Gelatinase B (MMP-9)

Stromelysines

1. Stromelysin 1 (MMP-3)
2. Stromelysin 2 (MMP-10)
3. Stromelysin 3 (MMP11)

Membrane-type metalloproteinases (MT-MMP)

1. MT1-MMP (MMP-14)
2. MT2-MMP (MMP-15)
3. Others: MMP17, 24 and 25

Others

MMP 7, 12 and 20

The inhibitors of MMPs are the Tissue Inhibitors MMPs (TIMPs)

Four different TIMPs have been identified in tissue (TIMP-1, TIMP-2, TIMP-3, TIMP-4)

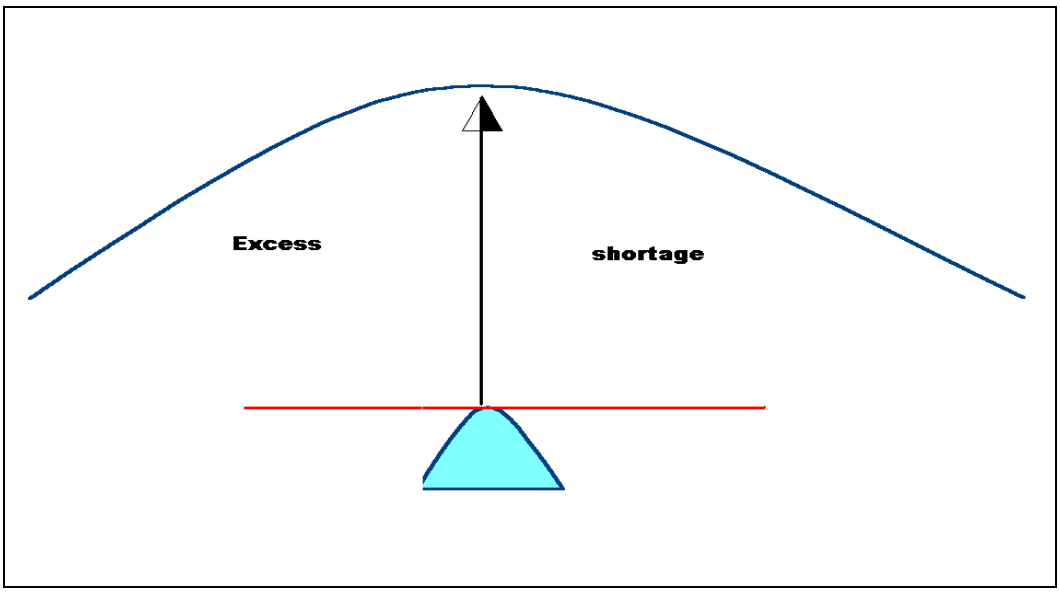
- TIMPs can inhibit all of the MMPs by binding to the zinc-containing active site of the enzyme
- The same cells that produce MMPs synthesize TIMPs
- In normal wound repair a delicate balance exist between the activity of MMPs and TIMPs.

Over expression MMP-2 by the fibroblasts

High MMP-2 production leads to break down of collagen type IV, laminine, fibronectine and therefore to basal membrane instability.
High MMP-2 production also facilitates metastasis of malignancies

The Art of Balancing MMPs

Balance between MMPs & TIMPs is essential to reach successful wound repair within a reasonable time frame



Normalization of the wound environment by controlling the balance of the MMP metabolism is crucial in the treatment of non-responding chronic wounds.
Most non-responding wounds will heal as soon as MMP levels are brought back into balance

3. DIFFERENT TYPES OF CHRONIC WOUNDS

3.1. DIABETIC FOOT ULCER (DFU)

Definition

Infection, ulceration and/or destruction of deep tissues associated with neurological abnormalities and various degrees of peripheral vascular disease in the lower limb.

Epidemiology of the diabetic foot

More than 120 million people in the world suffer of diabetes mellitus.
The major adverse outcomes of diabetic foot problems are foot ulcers and amputations.
Approximately 405-60% of all (non) traumatic amputations on the lower limb are performed on patients with diabetes.
85% of diabetes-related lower extremity amputations are preceded by a foot ulcer.
Four out of five ulcers in diabetic subjects are precipitated by external trauma.
The prevalence of foot ulcer is 4% to 10% of the diabetic population.
The prevalence of foot ulcers in developed countries has been estimated to be approximately 4%-10% of diabetic patients.

In diabetic foot ulcers glucose inhibits proliferation of endothelial cells and angiogenic mediators are deficient.

Laser Doppler Perfusion Imaging
A laser Doppler perfusion imaging is a noninvasive method for investigating skin microvasculature. A two dimensional flow map of specific tissues and visualization of the spatial variation of perfusion can be created with this technique.

Classification of Diabetic Ulcers

Diabetic ulcers can be classified by the depth of damage.
This classification is called the Wagner-classification

Grade 0 – no ulcer, high risk foot
Grade 1 – superficial ulcer, mostly located at the heads of the first metatarsal
Grade 2 – deep ulcer, involvement of underlying tendons
Grade 3 – deep ulcer, involvement of underlying bone and eventually causing osteomyelitits
Grade 4 – digital gangrene as a result of infection/vasculitis
Grade 5 – extensive necrosis of the total foot due to failure of arterial flow

Factors associated with foot ulcer
A. neuropathy (damaged nerves)
B. peripheral vascular disease
C. combination
D. trauma
E. previous ulcer/amputation
F. biomechanics
G. socio-economic status

Secundairy complication is an infection

Neuropathy = most important cause of diabetic ulcers
- Sensory
- Motor
- Autonomic

Secundairy complication: infection

Sensory neuropathy
- Loss of pain
- Loss of pressure awareness
- Loss of temperature awareness

Motor neuropathy
- Results in atrophy
- Weakness of the muscles of the foot
- Flexion deformity of the toes
- Abnormal walking pattern
- Deformities lead to areas of increased pressure

e.g. under the metarsal heads and the toes

Autonomic neuropathy
- Results in reduced or absent sweat secretion
- Dry skin with cracks and fissures
- Increases flow through arterio-venous shunts
- Warm, edematous foot

Peripheral vascular disease
- Thickening of the basement membrane
- Endothelial swelling of the capillaries
- Minor edema can result in total occlusion of
- Already compromised end arteries (gangrene of the toe)

Biomechanics
- Limited joint mobility - due to glycation of proteins in joints, soft tissue and skin
- Bony prominences
- Foot deformity
- Callus

General management of diabetic foot ulcer
- Improve circulation – vascular surgery
- Treat edema
- Treat infection – culture / antibiotics
- Improve metabolic control
- Non-weight bearing
- Foot surgery
- General condition
- Topical treatment
- Education

Topical treatment of diabetic foot ulcer
- Debridement
- Dressings – stimulation re-epitheliliazation with DerMax
- Topical agents
- Skin grafting

3.2. **DECUBITUS**

Definition
A pressure sore is an area of ulceration over a bony prominence, which has been subject to prolonged pressure and shear forces from any surface, including a bed or chair.

The term “pressure sore” is associated with bed sore. However, they do not only occur in patients who are confined to bed.
They are most common in patients who have been immobilized for a long time: the elderly, paraplegics, sedated mentally ill and people recovering from orthopedic surgery. It is the immobility of these people that leads to the lack of blood to the area under pressure. Such an area is termed “ischemic” i.e. lacking in an adequate blood supply. When the blood supply is cut off cells die due to a lack of oxygen and nutrients and because metabolic waste products are not carried away.
The blood supply only needs to be cut off for two hours for sufficient damage to occur to cause a pressure sore.

The major factors which contribute to the development of a pressure sore are:
- Pressure
- Friction
- Shearing forces
- Moisture causing maceration of the skin, especially urine

Classification of Pressure Sores
Pressure sores can be classified by the depth of damage into 4 stages.

Stage I
Characterized by an area of hyperemia = redness
Results from an acute inflammatory response
Is reversible

Stage II
Superficial break and is equivalent to partial-thickness wound

Stage III
Characterised by full-thickness damage and penetrates through the dermis to the subcutaneous fat layer

Stage IV
Extension through both skin layers and extends down to tendon, muscle or bone

General treatment per stage

Grade 1

Reduction of pressure and shear forces

- Prevention
- Skincare
- Treat malnutrion

Grade 2

Protection

- Blister protection – hydrogel / film dressing
- Drainage exudate - foam
- Re-epitheliliazation - DerMax

Grade 3

Debridement

- Enzymatic debridement
- Surgical debridement
- Drainage exudate – foam
- Re-epitheliliazation – with DerMax

Grade 4

Debridement

- Enzymatic debridement
- Surgical debridement
- Drainage of exudate – foam
- Stimulation granulation
- Stimulation re-epitheliliazation - DerMax

3.3. Leg ulcers

Definition

A chronic ulcer which is slow to heal, localized to the lower limb and caused by impaired circulation.

Depending upon the nature of the circulatory disturbance, different types of ulcers develop

Different types are:

- venous insufficiency - 72% to 81%
- arterial insufficiency – 12% to 31%
- mixed venous/arterial
- infections
- diabetes mellitus
- auto-immune
- maligne tumors
- vasculitis

The pathology of venous insufficiency - 72% to 81%

- Damage to valves of the deep veins and/or communicating veins
- Impairing return of venous blood
- Raised venous pressure
- Venous hypertension
- Deposition of fibrin extra vascularly → fibrin cuff
- Impede the exchange of oxygen and nutrients
- Deep venous thrombosis
- Superficial venous insufficiency

Venous insufficiency can result when the valves are damaged. The main cause of damage to valves is believed to be a deep vein trombosis which may have occurred years before an ulcer appears.

The clinical features of venous insufficiency are:
- Oedema
- Dry, often scaly skin
- Hyperpigmentation
- Induration = lipodermatosclerosis
- Large ulcer
- Seldom painful
- Surface ulcer greasy, seldom necrosis
- Secondary eczema
- Atrophie blanche = white atrophic plaques/purple papules/petechiae

Leg ulcers are slow to heal because the circulatory problems that cause the ulcer also affect the transport of leukocytes, oxygen and nutrients to the ulcer area.
The ulcers are usually relatively shallow and located above the ankle on the inside of the leg.

The pathology of arterial insufficiency – 12% to 31%
- Partial or total arteriosclerotic occlusion of an artery
- Intermittent claudication = pain in calf on walking
- High blood pressure
- Diabetes
- Smoking
- Smaller size of ulcer
- More in number compaired with venous ulcers
- Painful
- Surface of ulcer covered with yellow debris and/or black necrosis
- Surrounding skin→thin/hairless/cold

Vascular insufficiency occurs in arteries. Blockages known as arteriosclerotic lesions reduce blood supply to an area giving rise to ischemia and ulcerations.
These arterial ulcers are usually deep (extending into subcutaneous tissue) irregular, and usually located below the ankle. These ulcers are very painful and difficult to heal.

General treatment of a leg ulcer
- Correct diagnosis
- Correction circulatory disturbance
- Treatment of anaemia/vitamin C/zinc*/albumin deficiency
- Treatment of oedema
- Compression-therapy
- Wound care

*zinc in the tissue that is important (not the serum zinc)

4. **TREATMENT OF CHRONIC WOUNDS**

Selection of treatment depends on the type of the wound.
When you treat decubitus, reduction of pressure and slide forces are key. Also, when taking care of diabetic ulcers, “off-loading” is important, like compression therapy is to ulcus cruris venosum.

Depending on the phase in wound-healing, the choice for the right local therapy determines overall success.
When you look at the different stages:

- Necrosis needs to be removed
- Infections needs to be fought
- Granulation tissue needs to be stimulated

To this we can add three important pillars in treating chronic wounds:

1. Balance the Matrix metalloproteinases (MMP) production
2. Control Reactive Oxygen Species (ROS) production
3. Control bacterial contamination

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