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SKIN AND SOFT TISSUE LESIONS IN THE SURGICAL PATIENT THROUGH TRANSLATIONAL SCIENCES: PROSPECTIVE STUDY, EXPERIMENTAL APPLICATION OF THE TIME-H SCORE AND THE ROLE OF BIOLOGICAL THERAPIES.

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CHAPTER 1

Lesions of the skin and the impact of current research on Surgery

1.1. Introdution

The need for an updated scientific underpinning of the concern of wound healing in Surgery is without question. Papers published in recent years have undoubtedly improved the medical basis for this complex field. This, coupled with demographic modifications in many nations around the world, has led to growing numbers of sufferers developing wounds or wound healing problems. It's widely recognized in the majority of geographies globally that the variety of patients presenting wounds are increasing, in particular in Surgery.

There may be an ongoing shift inside the distribution of the sector's populace closer to old age, and we currently experience an increase in comorbidities like diabetes or cardiovascular insufficiency.

Specialists regularly care for patients with states of unusual injury healing, which include states like fibrosis, grips, and contractures, as well as states of deficient healing, like persistent non-recuperating ulcers, intermittent hernias, and surgical dehiscence. Notwithstanding numerous new advances in the field, which have featured the significance of subordinate treatments in boosting the mending potential, like enhancement of

therapeutic strategies, development factor treatment, progressed wound dressing materials, and bioengineered skin substitutes, states of unusual injury recuperating keep on causing huge expense, dreariness, and mortality.

1.2. Skin: anatomy and Physiology

Skin represents the most extended organ of the human body and it can be considered as a multifunctional station, with its several features:

- SENSITIVE FUNCTION, provided by nerve endings;
- PROTECTIVE FUNCTION, against physical damage, biological agents and radiations;
- TERMOREGOLATIVE FUNCTION, provided through regulation of blood flow and sweating;
- COMMUNICATIVE AND AESTHETIC FUNCTION;
- PRODUCTIVE FUNCTION, provided by the synthesis of Vitamine D.

Even though the skin and fascia contain a complex system of organs and anatomical characteristics, the layer arrangement is the most significant factor in wound closure. The epidermis, dermis, superficial fascia (also known as the subcutaneous or subcuticular layer), and deep fascia are the layers that make up the skin. If these layers are broken by damage, they should be thought of as planes that must be slowly and precisely reassembled. Each has its collection of characteristics that are critical for wound closing and healing.

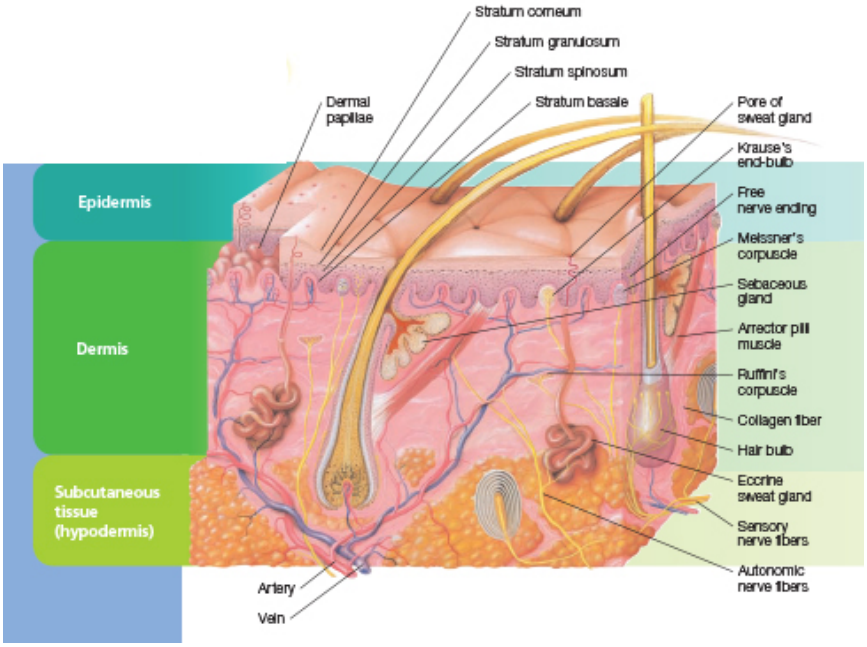

Figure 1.1. Cross section of the skin.

1.2.1. Epidermis

The epidermis is made up of five layers. Protrusions (known as rete pegs or epidermal ridges) reach down into the dermis from the innermost layer. These protrusions, which are surrounded by vascularized dermal papillae, protect the epidermis and enable fluid and cell exchange between skin layers.

The epidermis is composed of five layers:

1) Stratum corneum: it consists of dead cells and provides the maintenance of temperature and pH with an additional protective function. The continual replacement of the millions of worn-out cells contributes to proper skin repair.

2) Stratum lucidum: it is not always present in some anatomic areas of the body, where the skin is thinner. It provides extra protection in those areas exposed to wear and tear.

3) Stratum granulosum: starting from this layer, keratinocytes lose their cores and start to flatten, keratinization takes place in this layer. The stratum granulosum reduces the global loss of water from the epidermis.

4) Stratum spinosum: it contains living cells with spiny processes called desmosomes. The stratum spinosum is about 8–10 cells thick.

5) Stratum basale: it is also known as the *membrana basalis*, this is the lowest layer. It is one cell thick and forms a definitive border between the dermis and the epidermis. Cells at this level present as continually dividing and developing providing progressive rejuvenation of the skin. Melanocytes are produced at the level of this layer.

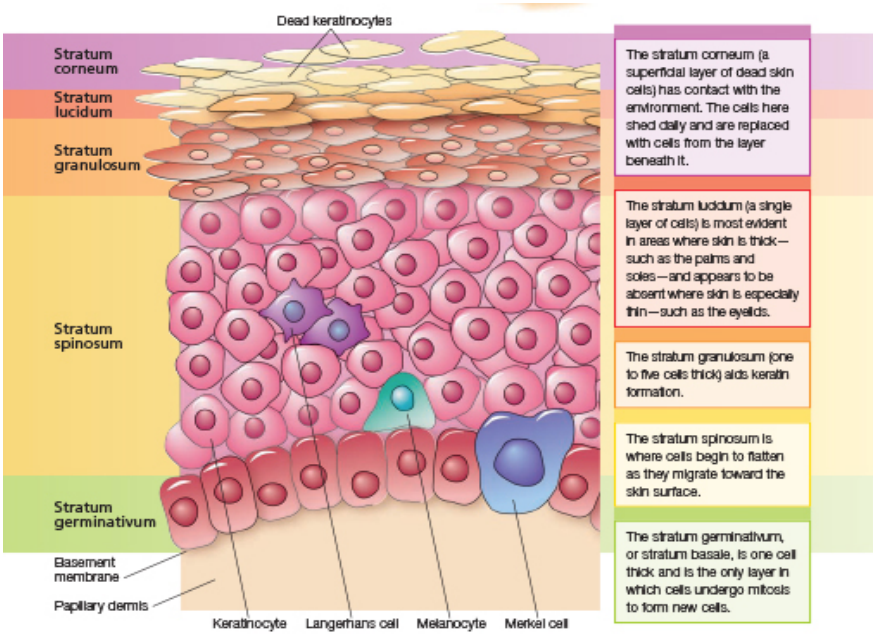

Figure 1.2. Epidermal layers

1.2.2. Dermis

The main role of this layer is to support and provide nutrition to the epidermis.

The core component of the dermis is proteinous connective tissue made up of arc-shaped elastic fibers, inelastic and undulated, collagen fibers. Other elements: mast cells, fibroblasts, other tissues cells, multiple blood and lymph vessels, nerve endings, hot and cold receptors, tactile sensory organs.

It contains blood capillaries, sensory nerve endings, lymphatic vessels, sweat glands, sebaceous glands and hair follicles.

The irregular tissue (made of elastin) and collagen fibers are rich in blood vessels, nerve fibers and lymphatics. Ridges from these bundles of collagen run downward, horizontally and forward around the body: they are genetically determined, unique for each person and named cleavage lines.

1.2.3. Hypodermis

The superficial facia provides anchorage to the skin while allowing some ability for it to move. It also offers support to the dermis and is made up primarily of connective tissue, adipose tissue and vessels. The fat stored

within the hypodermis offers protection to the internal structures from insults and against the cold.

1.2.4. Appendages

Hair

Composed by keratin, at the lower end, a bulb or root is enclosed in a follicle that produces the hair. The root is indented by a hair papilla, vessels and connective tissue. The hair follicle is an epithelium lined sheath, while the *erector pili* (smooth muscle) extends through the dermis and attaches to the base of the follicle, the hair stands on end when the muscle contracts.

Palm sides of the hands, soles of the feet, nails, portions of the external genitals, lips and nipples do not present hair. The main function is represented by protection. Protection of the skin by hair is constrained; however, its role is to protect the scalp specifically from ultraviolet rays, heat loss and injury. The eyebrows and eyelashes provide protection from foreign bodies entering the eye.

Nails

The nails are specialized types of keratin and are situated over the distal surface of fingers and toes. The nail plate is surrounded on three ends by cuticles.

The function of nails is to assist with the development of fine motor skills such as grasping, scratching and manipulation. It provides protection against trauma to the fingers and toes.

Sebaceous glands

These are located on all parts of the skin except palms; they are more prominent on the scalp, face, upper torso and genitalia, producing sebum, made up of keratin, fat and cellulose debris. Sebum forms a moist, oily acidic film that has antibacterial and antifungal properties.

1.2.5. Blood vessels

Blood vessels include arterioles, capillary networks and venules. Blood vessels in the skin are responsible for the transportation and distribution of oxygen, nutrients and hormones as well as the removal of waste products.

Nerve fibers

Within the dermis, there are both sensory and motor nerves. The sensory nerve endings are sensitive to touch, or initiate signals producing sensations of warmth, coolness, pain, pressure, vibration, tickling and also itching.

Lymphatic vessels

The lymphatic system strictly shares the blood vessels supply and function.

1.3. Cutaneous regenerative processes

Stages of wound healing are composed of a series of complex but orderly overlapping phases, in which highly specialized cells interact with the extracellular matrix, laying a new framework for tissue growth and repair. There are four different but overlapping stages of wound healing, including hemostasis, inflammation, proliferation and remodelling. These stages are affected by various cellular interactions and are regulated by the local release of chemical signals such as cytokines, chemokines, growth factors and inhibitors.

1.3.1. Hemostasis

Physiology of haemostasis

The haemostatic mechanism is complex and delicately balanced. Haemostasis is one factor of the wound restoration procedure. The wound healing procedure passes through some levels, which include infection, granulation and maturation.

Haemostasis is a manner that causes bleeding to prevent, keeping blood within a broken blood vessel; the opposite of haemostasis is haemorrhage, and it is the primary degree of wound healing.

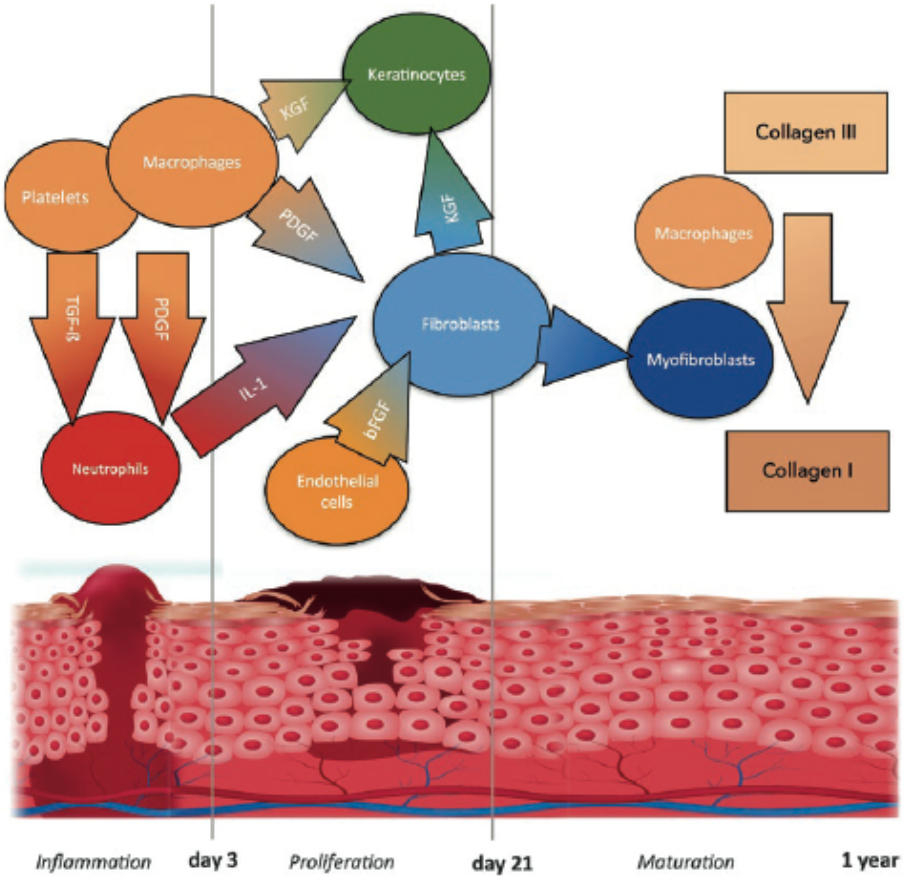

Figure 1.3. Phases of human adult wound healing.

The body's initial reaction to wounding is to control the lack of blood from the area. Following harm to blood vessels and endothelial cells, platelets become sticky and adhere to the wall of the blood vessel and each other, forming a platelet thrombus. The platelet thrombus acts as a temporary plug that reduces blood drift out of the wound. The platelets also release serotonin and other chemical mediators, which ends up in a brief length of vasoconstriction. Haemostasis is also performed by way of the activation of the clotting cascade, initiated through damage to the endothelium.

Platelet function

Platelet characteristic is important in haemostasis. Platelets are vesicle-like fragments; they may be incomplete cells, about 2–4 μm in diameter, discoid in form and rise from large cells, the megakaryocytes, within the bone marrow. The everyday platelet count number is approximately 3 × 1011/L.

Five different activities occur when there may be damage to a blood vessel:

- *local vasoconstriction*;
- *adhesion and aggregation of platelets;*
- *activation of the clotting cascade;*
- *activation of coagulation inhibitors;*
- *fibrinolysis.*

While harm to a blood vessel takes place, it causes it to constrict via direct movement and also circuitously via the release of vasoconstrictors from platelets; these movements play a principal function in limiting blood loss. Damage to the endothelial lining of the blood vessel also triggers platelet pastime; when this occurs the combination of platelets formes a plug. This, together with the temporary vasoconstriction is generally liable for the cessation of bleeding. Platelets while activated also unencumber several vasoconstrictor substances, revealing a phospholipid that is important for the introduction of a blood clot. Commonly, platelets do no longer adhere to the clean endothelial lining of the blood vessels, but, when the vasculature is broken, this exposes the blood to subendothelial collagen

and microfibrils. The platelets keep on with the collagen in the damaged vessel thru glycoproteins (GPs) (von Willebrand factor enhances adhesion) which can be positioned at the floor of the platelets. Activation results inside the platelets changing form along with the production of pseudopodia (these are transient protrusions) generating thromboxane A2 (TXA2) – an enzyme (a lipid with prothrombotic houses) – 5-hydroxytryptamine (5-HT or serotonin) and ADP (adenosine diphosphate). In addition, vasoconstriction happens due to TXA2 and 5-HT; ADP recruits extra platelets and the mixture to each different cross-linking with fibrinogen. A soft plug bureaucracy is held collectively by using fibrinogen molecules that shape bridges between adjacent platelets. The aggregated platelets occlude the wound, which ultimately stops bleeding.

1.3.2. Clotting cascade

The clotting cascade affords this extra stable consolidated plug and in the end consequences in the conversion of the plasma protein fibrinogen to fibrin, which paperwork a meshwork offering a seal to the damaged vasculature. The cascade is a sequence of interactions among proteins that reason fibrin depositions on the region of tissue injury and is initiated by its interplay with activated factor VII.

The preliminary phase (this was once known as the extrinsic way) includes numerous elements. Tissue factors VII and VIIa convert factor X to its lively

form, Xa. VIIa additionally covers aspect IX to its activated form, element IXa; further technology of component Xa is inhibited through the tissue component pathway inhibitor. At this point, the quantity of element Xa

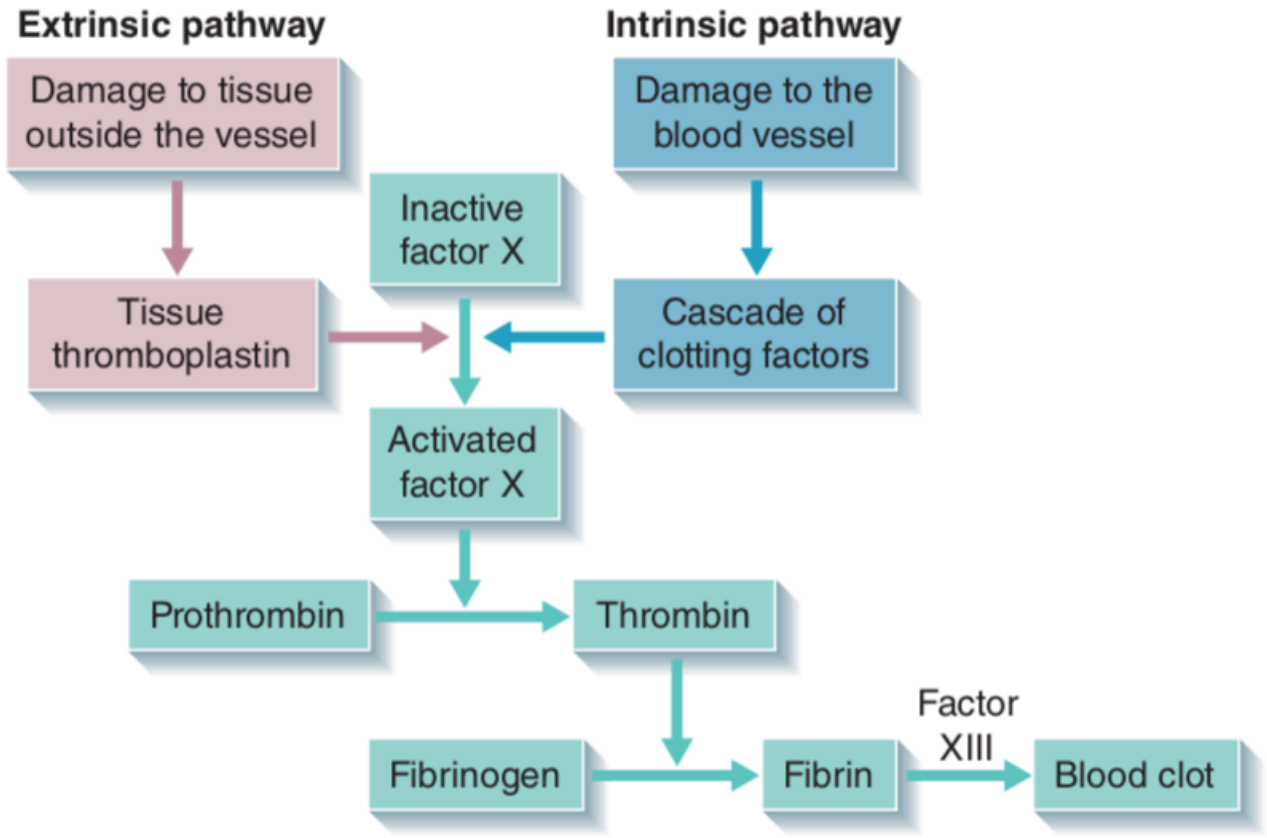

Figure 1.4. The clotting cascade.

produced is insufficient to preserve coagulation. Similarly, element Xa to permit haemostasis to progress to of entirety can now only be generated via the IXa factor pathway; through this level, though enough thrombin has been generated by the element Xa to set off factors VIII and V. Those both act as mighty catalysts. VIIIa will increase the capability of IXa to prompt factor X to Xa many thousand times. The elevated component Xa produced in this manner with its cofactor, activated Va, forms a complex that

promotes the green conversion of prothrombin to thrombin. Thrombin then lets in the conversion of fibrinogen to fibrin. The very last result of the cascade is the production of fibrin, the biological glue that sooner or later seals the haemostatic plug making sure haemostasis. As quickly as a small quantity of thrombin is formed the clotting system hurries up and gives increasingly more thrombin into the wound. At excessive concentrations, this thrombin quickly converts fibrinogen to fibrin on the surface of the platelet aggregate to stabilize the haemostatic plug. Over the route of the subsequent 7–12 days, the manner of fibrinolysis dissolves the fibrin within the wound because the website online of damage heals and the mobile layer in the vessel wall is restored. Scar formation happens, and inside some weeks the wound is healed.

1.3.3. Inflammation

When there is an insult to the body, for example, trauma or intentional damage resulting in harm to the blood vessels the first reaction is to arrest a haemorrhage. Prevention of blood loss and the formation of clots and scabs represent a try to offer a defensive covering while the pores and skin damage are part of the haemostatic system.

The human body has powerful mechanisms to block ability pathogens from entering the frame, together with the skin and mucous membranes as physical limitations. Enzymes consisting of lysozyme in tears and sweat

have the potential to wreck many capability pathogens chemically; but, despite these external barriers available to fight potential pathogens, events arise when pathogens enter the body. When this occurs and contamination is present the body sets into movement the inflammatory reaction. This response is non-unique and attacks foreign invaders. This inflammatory reaction is a time-regulated reaction to tissue damage.

Irritation attempts to rid the frame of microbes, pollutants or different overseas cloth at the site of harm to prevent they are unfold to different tissues; it additionally starts to put together the website online for tissue restore.

The inflammatory response

Inflammations represent an innate reaction to tissue harm, with four levels (or feature signs) associated with the inflammatory reaction:

- *redness (rubor)*
- *swelling (tumour)*
- *heat (calor)*
- *pain (dolor).*

Inflammation can result in loss of function; for example, as a result of the lack of ability to stumble on sensation relying on the site and extent of the injury.

When damage occurs pathogens including organisms, viruses or fungus advantage entry to the frame. Almost without delay the broken cells purpose some of the activities to appear:

- *vasodilation*
- *messenger molecules are released*
- *activation of supplement*
- *extravasation of vascular additives*
- *phagocytosis*
- *ache.*

The mast cells injured inside the connective tissue release histamine. The arrival of histamine on the website of harm has a right away effect on blood vessels inside the place. Arterioles dilate, venules constrict, causing a boom in blood flow. Vasodilation is introduced about by using four essential mechanisms.

1 The kinin system within the cell produces bradykinin a vasodilator, also responsible for pain.

2 Damaged plasma membranes release arachidonic acid, a fatty acid, a precursor to prostaglandins. Prostaglandins also are vasodilators and feature hyperalgesic homes (they boom ache).

3 Release of histamine from the degranulated mast cells will increase pore length between the capillary cells, allowing motion of proteins and different molecules into interstitial areas.

4 Vascular epithelial cells launch nitric oxide, a vasodilator. Macrophages additionally release huge amounts of nitric oxide.

Improved blood flow explains redness and heat associated with contamination. Capillaries within injured tissue dilate, turning into permeable; this is crucial for the correct inflammatory response, offering a

possibility for some of the blood components to be released into the broken location and the site of contamination.

Platelets and clotting factors

Platelets and their related clotting factors go out thru the leaky capillary walls and migrate in the direction of the website of harm. The clotting factors serve a dual purpose while this occurs, they help to plug the wound and seal broken blood vessels.

Chemokines

The second line of defence has additionally been activated. Cells near the injury launch a series of chemical indicators radiating from the website online of inflammation; those alerts are called chemokines. Awareness of chemokines is finest right away surrounding the contamination. High stages of chemokines attract or provide a sign for the enchantment of phagocytic white blood cells including neutrophils.

Phagocytosis

As chemokine awareness maintains to boom, phagocytes leave the capillary coming into the site of infection macrophages to arrive about 24 hours later. Phagocytes engulf and spoil the pathogens pre-despatched, recognizing the pathogen as non-self remember and send out pseudopodia sounding the pathogen. The wound starts to heal. After the white blood

cells engulf pathogen particles, they begin to die; they eventually make up the pus associated with infected cuts. The important thing molecule launched is interleukin 1, attracting neutrophils and macrophages to the site of harm, helping to clear away debris from the injured vicinity.

Phagocytosis outcomes in a metabolically intensive interest and is chargeable for some of the heat associated with irritation. Pyrexia occurs during contamination accompanying irritation. Bacterial pollution elevates body temperature, releasing cytokines from macrophages inflicting temperature growth. The presence of pyrexia exaggerates the impact of interferons, hindering the growth of a few microbes dashing up reactions that resource repair.

With the pathogen particles destroyed and damaged tissue repaired, histamine indicators fade, and blood vessels return to everyday length.

Ache is generated through action and interaction of bradykinin and prostaglandins, growing the feeling of ache in irritation. Different chemical substances are also worried about the stimulation of pain after the injury has occurred; lactic acid produced with the aid of anaerobic mobile respiration is one instance. Hydrogen ions and potassium released from broken cells also stimulate the ache receptors.

Inflammation lasts for approximately 4–five days. The system calls for electricity and dietary sources for efficacy. Inflammation has a protecting characteristic supporting to take away the reasons for tissue harm. Extending the inflammatory degree, for instance, in which there may be infection, the presence of an overseas frame or damage that has been

because of an inappropriate dressing, could hurt the individual's fitness and wellness.

1.3.4. Proliferation

The proliferation overlaps with the infection level as this segment starts to end. The focal point of the proliferative section is related to the construction of the latest tissue to fill the wound area. Because the infection diminishes, work starts to restore the injury. About three days after damage, fibroblasts will start to go into and assemble in the wound; this is the beginning of the transition from inflammatory section to proliferation section. The fibroblasts are connective tissue cells that synthesize and secrete collagen in addition to the secretion of increased factors that result in the growth of blood vessels through the manner of angiogenesis and at the equal time promoting endothelial cell proliferation and migration. Because the fibroblasts grow up, they produce a new, provisional extracellular matrix that has come about through excreting collagen and fibronectin.

The fibroblasts and endothelial cells form granulation tissue that acts as the inspiration for scar tissue development.

1.3.5. Remodelling

As soon as harm befell, cytokines are launched from platelets and they activate keratinocytes. As the migration of keratinocytes occurs, re-epithelialization begins as early as 2 hours after wounding. Key elements along with keratinocytes growth component (KGF) and epidermal growth factor (EGF) provoke proliferation and migration of keratinocytes. This phenomenon, over the wound web page, is likewise stronger via loss of contact inhibition and release of nitric oxide from polymorphonuclear leucocytes (PMNs), keratinocytes and fibroblasts. Epithelial cells hold migrating across the wound bed till cells from extraordinary facets meet in the centre, at this factor touch between keratinocytes inhibits similarly migration. New layers of keratinocytes differentiate and this gives rise to a stratified epidermis.

As the proliferative degree ends white blood cells leave the place and oedema diminishes and the wound starts to blanch because the small blood vessels start to thrombose and degenerate.

The maturation and remodelling stages overlap with the proliferation segment as healing begins to come back to an give up. The remodelling phase starts after approximately three weeks and can preserve for six months or longer. Final scar tissue begins to form through the simultaneous synthesis of lysis and collagen. Collagen is fibrous in person, connects and supports tissues and organs including skin, bone, tendons, muscles and cartilage. It's far frequently called the glue that holds the body together; it's miles collagen that gives tensile electricity. There are over 25

sorts of collagens that occur evidently inside the frame; it can be discovered both outside and inside cells, contributing to the shape of cells. At this stage, the system of remodelling of the collagen fibres is laid down. The scar turns into avascular.

The wound is made smaller by using the motion of myofibroblasts; as the edges of the wound are drawn closer together this establishes a grip on the edges of the wound, inflicting them to contract using a mechanism that is just like that during clean muscle cells. whilst the position of myofibroblasts is near the final state, cells that can be not needed undergo apoptosis. A myofibroblastic pastime can persist, contributing to fibrosis and scarring within the skin.

Nerve endings at the moment are redeveloping and the tissue begins to arrange itself. Scar tissue may reap seventy–eighty % of tensile power with the aid of the give up of three months.

Speedy keratinocyte migration at the side of re-epithelialization can often result in better wound-recuperation outcomes and reduced scar formation. However, exposure to air and loss of moisture will bring about a put off inside the recuperation procedure.

This whole process is complex and the skin is fragile and prone to interruption or failure and the outcome of this is the formation of non-recovery chronic wounds. There are factors that can contribute to interruption or failure and these consist of diabetes, venous or arterial disorder, contamination, growing old.

CHAPTER 2

Surgical Skin lesions: clinical scenarios and contemporary research

2.1. Skin lesions – classification

Identifying the cause of a cutaneous ulcer is challenging process. It requires, not infrequently, a global expertise in surgery, dermatology and a thorough laboratory investigation.

A well-known axiom in medicine says that things do not always turn out to be as they appear at first sight. A cutaneous ulcer that seems to be linked to a venous insufficiency may, following thorough investigation, reveal to be a manifestation of carcinoma or the result of a hidden infectious process. In some patients, the underlying disease is rare, requiring evident clinical experience for its identification.

For many patients several co-existing etiologies can be found. Sometimes, in some clinical conditions, such as lymphedema, venous insufficiency, peripheral arterial disease, or diabetes, the skin seems to result more vulnerable. In these cases, the development of a cutaneous ulcer is not necessarily spontaneous, because the skin tends to ulcerate following several triggers such as mechanical injuries, blunt trauma, or contact dermatitis. The physician should distinguish between underlying

conditions that gradually affect the quality of the skin and random or definite triggers that may result directly in ulceration.

2.1.2. Origins of Skin lesions

In clinical practice it is possible to find a wide array of etiologies of skin lesions. However, in many patients, it is not enough to simply classify an ulcer as being caused by a specific factor. In a known disease, or for any given etiology, the lesion may be characterized by a series of complex mechanisms. The exact etiology by which the ulcer was caused should also be considered.

The unexperienced physician may assume that the main mechanism of ulceration in an infectious process, for example, should be the direct toxic effect and its subsequent abnormal local inflammatory processes. But clinical evidence shows how some infectious diseases may result in skin lesions by other mechanisms. Hepatitis B may lead to cryoglobulinemia, vasculitis, or periarteritis, all of which may conduct to the development of skin lesions.

Similarly, a lot of pathways may conduct to skin diseases characterized by connective disfunctions.

2.1.3. Mechanical trauma and Skin lesions

Mechanical trauma and other types of injury may finally result in a cutaneous loss of substance. In several cases, the external trauma may be of minor entity. Although the patient may consider it to be the origin of the ulcer, the cutaneous layer may have been previously interested by some underlying process (f.e., aggressive fungal infections or malignancies) which may well represent the actual cause of the lesion and should be quickly identified.

It's far vital to underline that skin losses of substances may evolve right into a portal of access for fungal or bacterial infections, with subsequent ulceration. Additionally, a superficial erosion connected to trauma may become an ulcer following bacterial infection. The possibilities of this happening are much better whilst it can be identified an underlying hassle together with diabetes or peripheral arterial disorder.

In most instances, self-inflicted ulcers are precipitated through continuous scratching, rubbing, or slicing of the skin. Once an ulcer exists, the persevering with fiddling with it with the aid of the affected person interferes with its everyday recovery method.

The medical appearance of such ulcers depends on the manner wherein they have been created. In some instances, self-inflicted ulcers are prompted by way of injecting foreign bodies into the skin.

2.1.4. Contact Dermatitis

Patients presenting leg ulcers non rarely suffer from contact dermatitis. Ulcerations of the leg have been reported following enduring exposure to several topical preparations applied to cutaneous ulcers, like topical antibiotics, some vehicles (lanolin), or preservatives. For those patients, the application of these preparations could significantly aggravate the clinical situation of ulcers. Topical treatment should be re-evaluated and patch tests performed, when requested. Moreover, skin areas affected by contact dermatitis may be secondarily infected, with the subsequent formation of cutaneous ulcers.

2.2. Surgical skin lesions

The clinical classification of skin lesions affecting the surgical patient should be placed into two main categories: *acute* and *chronic*. There are also two subcategories that focus on the phases of the wound healing process and the tissue types on the wound bed state at any given time. The chart of wounds enables the physician to accurately identify and provide the holistic assessment of the patient. It therefore may become clear on clinical evaluation of the patient that an acute wound will undoubtedly become chronic for the underlying co-morbidities for some patients.

2.2.1. Acute wounds

An *acute wound* is induced by surgery or trauma. There can be identified many different causes of trauma in the large surgical everyday field:

Incision. Also described as a 'surgical cut'; this kind of wound is usually caused by a sharp object (f.e. scalpels, knifes, shards of glass or a metal sheet) that leads to a slice to the skin. There is usually very small tissue loss of substance and the edges are usually very clearly defined. The depth can range from superficial to deep.

Laceration/skin tear. This is generally linked to a blow against a blunt object that causes the skin layer to split. Sometimes there is swelling and some additional tissue loss. It often creates a skin flap that can be identified as very thick or very thin in depth. A skin tear is usually originated by the tearing of the skin by a sharp object or by clothing/belts or rough handling. The frail elderly skin is often subject to this type of trauma. The depth is usually superficial and confined to the skin, but can affect deeper tissues.

Burn. It can be caused by heat (fire), cold, chemicals and electricity. It is fundamental to find the cause of the burn to treat it appropriately. It can range in depth from superficial to deep.

Friction. This is the erosion of superficial (but also deeper) tissue layers originated by the sudden or constant rubbing of the skin layer against a rougher surface. This kind of wound requires healing by secondary intention.

Shearing. This is represented by a closed wound in which tissues attached to the bone layer are torn away from the bone itself by the opposing forces of two types of tissues. The affected parts are deep seated and can be clinically painful due to inflammation of the bursa at the location of trauma. This kind of injury is not always visible to the naked eye, but it will make the patient more vulnerable to rapid onset of pressure damage.

Puncture. This is a penetrating lesion that can be clinically identified as varying of depth, caused by pointed objects like wooden stakes, needles, pins teeth (f.e. dog bites). These can appear insignificant for the small opening on the skin layer, but the underlying potential structural damage and infection represent a consistent risk with this wound type. The broken bones can also originate this kind of lesions.

Scald. It can be caused by steam or liquids and it must be quickly cooled. The resulting damage can vary in depth from superficial to deep.

Contusion. This represents a bruise originated by a rupture of superficial blood vessels due to the trauma, with no break to the skin layer. The bruising will end in around two weeks thanks to the venous and lymphatic drainage systems. The darker the discolouration clinically presents, the deeper the damage is usually located.

2.2.2. Chronic wounds

A chronic wound is potentially induced by several causes and does not progress through the classic phases of wound healing ending into a prolonged or static wound due to original causes, usually of a duration longer than 40 days. The following lesions may become chronic, but any loss of substance from any cause can become chronic due to underlying factors that affect the healing chances: pressure sores, diabetic foot ulceration, leg ulcers, several skin conditions.

2.2.3. Host Defense Peptides and Chronic Wounds

Host defense peptides represent short (about 12–50 amino acids) cationic polypeptide sequences that present antimicrobial and immunomodulatory properties and are produced by all complex life forms. Larger proteins (more than 100 amino acid residues) are also sometimes included in the definition of HDPs f.e. lactoferrin, calprotectin (S100A8 and S100A9), psoriasin (S100A7), RNAse 7, and lysozyme. Originally appreciated for their direct antibacterial activity toward microbes, they are usually referred in the literature as antimicrobial peptides (AMPs). However, subsequent studies of these kind of molecules have revealed that these peptides exert a wide range of immunomodulatory targets, which might indeed be their main function in the human body. These include cell recruitment or

chemotaxis, antiendotoxin activity, modulation of chemokine and cytokine production, angiogenesis, leukocyte activation, and wound healing properties. It is for this reason that contemporary research adopt the term HDP to better encapsulate the breadth of biological functions mediated by these molecules. More recently it has been shown that a distinct subset of HDP also have preferential anti-biofilm activity.

In humans, HDPs are produced by several cell types throughout the body. Immune cells like monocytes, neutrophils, lymphocytes, natural killer (NK) cells, and mast cells all produce and store various HDPs. The innate immune response is deeply linked to the presence of these cells to release HDPs in response to an invading pathogen and to avoid the onset of an infection. Several HDPs are also brought by the epithelial cells of healthy skin, and it is thought that the presence of these peptides on the skin surface helps maintain homeostasis with the skin microbiota and prevents colonization and potential infection by invading microbes. Important types of HDPs present in healthy human skin include RNase 7 and psoriasin, dermcidin, hBD-1 and lysozyme. The expression of many HDPs is upregulated upon skin wounding, which indicates that they might play an important role in the whole wound healing process. For example, the human cathelicidin HDP (LL-37) is upregulated in the skin in response to inflammation, like in case infection by group A Streptococcus. A more recent study found that injury of the human epidermis of the skin alone was a major booster of a wide range of HDPs, including human β-defensin

2 (hBD-2) and hBD-3, like various cytokines, such as interleukin (IL)-6 and IL-8.

The central role of natural HDPs in wound healing has been extensively summarized in several reviews. The upregulation of gene expression for these molecules leads to an increase in the local concentration of HDP which, if large enough, can directly kill bacteria and prevent important infection. In addition, HDPs are known to interact with various cells of the immune system, like epidermal keratinocytes, to promote the wound healing process. These activities include activation of cytokine production, promoting cell migration and proliferation, and blood vessel formation, all of which are linked to the wound healing process.

There is global evidence that the dysregulation of endogenous HDP levels contributes to impaired wound healing and chronic infections. For instance, patients with atopic dermatitis have been shown to have reduced expression levels of hBD-2 and LL-37 in skin interested by inflammation, and a marked reduction of hBD-2 expression has been found in burn wounds, both of which may be the spy for the grown susceptibility to bacterial infections in these patient groups. In the context of chronic wounds, LL-37 levels have been found to be low near the wound edge side of chronic ulcers, while hBD-2 levels are represented as upregulated in or venous ulcers or diabetic foot.

2.2.4. Current translational researches perspectives: synthetic HDPs as new wound healing agents

Synthetic derivatives of natural HDPs have been demonstrated to retain many of the biological properties of this kind of peptides, and in some cases, peptides presenting enhanced action or lower cytotoxicity have been studied. Most of these translational research strategies have been aimed at identifying HDPs with improved antimicrobial activity. This type of study has dramatically let grow our knowledge of AMP sequences and has expanded the breadth of sequences that are known to have inside antibacterial activity. These sequence optimization strategies have also been brought to other HDP kinds of activity, suggesting that it may be possible to optimize synthetic peptides for specific biological activities. Fragments of LL-37 have been created for the retain of antibacterial potency and chemotactic activity of the parent peptide, while showing reduced cytotoxicity. Of importance to chronic wounds, several synthetic peptides with anti-biofilm activity have also been studied by our group, and it appears that this activity is not linked to a direct antibacterial activity toward planktonic cells. Therefore, an optimization technology aimed at enhancing the anti-biofilm potential of synthetic peptides could address the biofilm component of a chronic lesion that is not specifically reached by conventional antibiotics molecules alone.

Furthermore, the wound healing properties of native HDPs have also been revisited in synthetic peptides. For instance, a model-derived AMP,

esculentin-1a, stimulated migration of keratinocytes more quickly than LL-37 in vitro. Promotion of wound healing has also been shown in vivo by IDR-1018, a synthetic molecule of the bovine HDP bactenecin, which enhanced wound healing in S. aureus-infected mouse wounds. A recent study described the wound healing characteristics of DRGN-1, an animal histone-derived peptide, in several species cutaneous infection model as well as in sterile lesions. New evidences suggest that improving synthetic peptides for specific wound healing characteristics could lead to novel peptide sequences with therapeutic potential. Nakagami et al. studied a series of derivatives based on a new angiogenic peptide molecule, AG30, to try to support both the angiogenic and antibacterial properties. One of these derivatives, AG30/5C, in which five parts in the parent sequence were replaced with Arg or Lys fragments, promoted wound restoring and new angiogenesis in a diabetic mouse wound model infected with MRSA. This peptide has then been subsequently optimized to peptide SR-0379 by identifying the minimal peptide sequence required for wound healing as well as incorporating a D-Lys residue near the C-terminus to support the proteolytic stability and avoid toxicity.

The wound healing properties of HDPs do not appear to be directly linked to the antibacterial characteristics of a known peptide sequence. HB-107 represents a portion of the insect AMP cecropin which has not microbicidal activity but promotes wound restoration in mice and improves keratinocyte hyperplasia and leukocyte migration in wounds. This kind of observation suggests that several biological activities influenced by HDPs

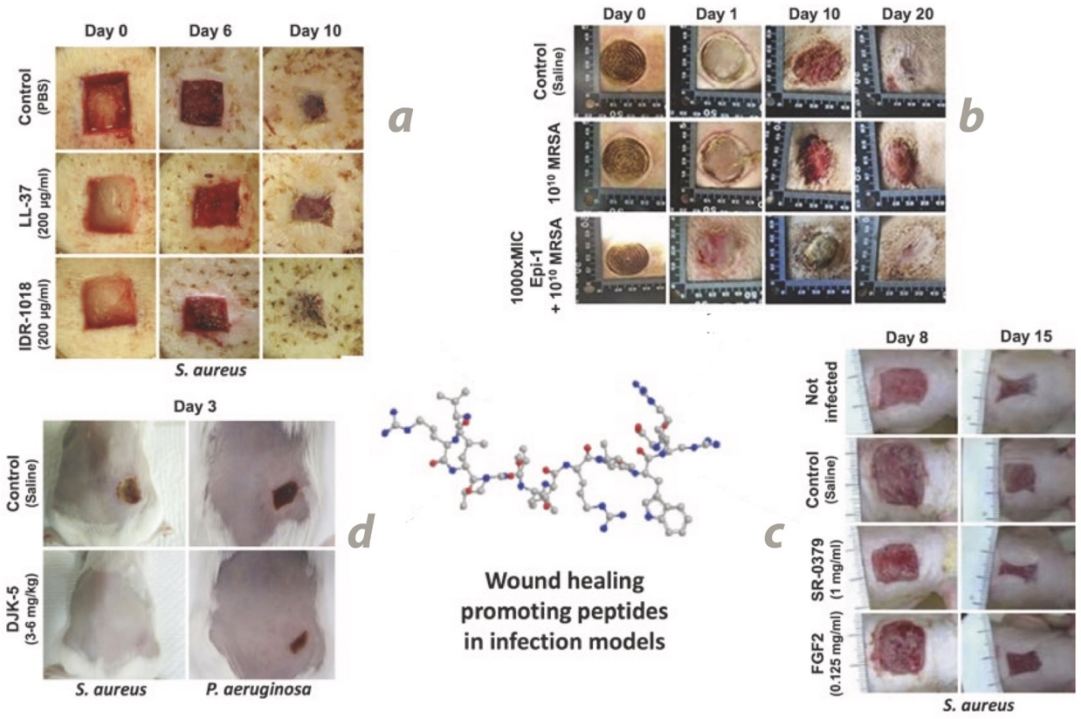

Figure 2.1. Wound healing-promoting peptides in infection models. A. S Aureus-infected Porcine wound healing model. B. S Aureus-infected burn wound swine model. C. S Aureus acutely infected wound compared to saline control treatment as well as uninfected wounds. D. Anti-biofilm peptide DJK-5 in comparison to a saline control in a high-density bacterial infection model in CD-1 mice which were subcutaneously injected with S Aureus or P Aeruginosa.

are sequence specific. It also shown that the antibacterial properties of AMPs do not directly correlate with anti-biofilm activity. LL-37 and the synthetic peptides 1037 and IDR-1018 avoid biofilm growth at standard concentrations. IDR-1018 acts against biofilms of Burkholderia Cenocepacia, which is completely untouched by to AMP activity, while one of the most potent AMPs did not show anti-biofilm activity. With the help of the "best" anti-biofilm peptides, this activity is potentially broad in spectrum, preventing biofilm formation and killing preformed in vitro bio-films caused by all of the known nosocomial antibiotic-resistant (also called ESKAPE) pathogens, destroying multispecies oral biofilm bacteria, and acting against in vivo biofilms. Furthermore, it is interesting to ipotyze that it may possible to control specifically synthetic HDPs with enhanced wound

healing properties, growing anti-biofilm activities, and anti-inflammatory activity to improve the contemporary Translational Research concerns for chronic wounds.

Several in vitro screening methods are described to evaluate synthetic peptides for wound restoring action. The most frequent are represented by cell proliferation assays, looking primarily at sequences that support fibroblast and epithelial cell growth, and migration assays to quantify movement of epithelial cells, such as keratinocytes, across a surface. These practices are essential during the proliferative stage of wound healing as fibroblasts release essential components at the level of the extracellular matrix and keratinocytes migrate to re-epithelialize wounds. Many synthetic HDPs with wound healing properties were characterized starting from these types of assays *in vitro* prior to *in vivo* works. Some groups are already trying to find new wound healing peptides starting from these screening approaches. Kosikowska et al. tried to identify bifunctional AMP sequences characterized by potent antimicrobial properties with enhanced cell proliferation and migration properties. In this study, while the authors identified peptides with antimicrobial activity or wound healing potential, they did not identify a peptide that fulfilled their main focus. It has been successfully used a strategy to simultaneously evaluate the immunomodulatory and anti-biofilm activity of peptide sequences and generated optimized synthetic HDPs for several activity types. As the Translational Researches of the wound healing properties of HDPs improve, it should be possible to further support the therapeutic potential

of these molecules and advance their progress to the clinic everyday practice.

2.3. Infections management

Several injuries may be aggravated when they finish to be secondarily infected by bacteria such as Streptococcus or Staphylococcus. For some patients, an ulcer caused by a relatively minor trauma may become deeper as a result of infection.

Focusing on some critic anatomic areas, limbs affected by venous insufficiency, diabetes, or peripheral arterial disease, are at a particularly high risk of developing lesions following trauma, which may be associated with infection. By the same main concept, erosions of bullous diseases may be in the case of a secondary infection, resulting in the formation of cutaneous losses of substance.

2.3.1. Cellulitis and Erysipelas

Cellulitis and Erysipelas may lead to leg ulcers. The main strategy of ulcer formation in these cases is represented by the direct effect of bacterial toxins. The associated edema also interferes with venous and lymphatic return and it also contributes to the formation of the lesion.

About 3% of cases of bullous erysipelas may end in ulceration. Other cases of wounds linked to cellulitis or erysipelas are caused by abscess formation, or concomitant trauma. It is also important to underline that cellulitis or erysipelas can be linked to a pre-existing wound.

Episodes of erysipelas of cellulitis potentially cause cumulative tissue damage, which day by day compromises lymphatic and venous return.

2.3.2. Necrotizing Fasciitis

Necrotizing fasciitis represents an extreme example of an ulcerative lesion caused by bacterial infection. It is identified as an infection of the subcutaneous tissue, causing invasive and massive destruction of the fascia and subcutaneous fat, with the additional presence of overlying cellulitis. In cases where the genitalia are involved, this disease is also known as Fournier's gangrene.

In the past two decades there has been a noticeable increase in reports of invasive group A streptococcal infections, leading to necrotizing fasciitis. Other kinds of bacteria, such as Clostridium Septicum or Clotridium Perfringes, can also lead to necrotizing fasciitis. It also may be caused by a synergistic combination of organisms like anaerobes with aerobic gram-negative bacteria. Mixed infections tend to occur in young people, in diabetic patients, or in patients presenting an open lesion exposed to bowel content.

The clinic evolution of necrotizing fasciitis is extremely severe, frequently with a dramatic fatal outcome. It can also occur in healthy people, sometimes following a relatively minor trauma or surgery. A high incidence of necrotizing fasciitis has been reported in people with obesity, diabetes, malnutrition, malignancy, and in drug addicts.

Its severe pathogenicity is linked to bacterial toxins like as exotoxin A in the case of group A Streptococcus. Delay in the diagnosis or treatment of this disease can bring to fatal consequences. It requires radical debridement, high-dose antibiotic therapy, and intensive care support.

2.3.3. Tuberculosis

Ulcers may occur in several types of tuberculosis:

Lupus vulgaris. Skin lesions of lupus vulgaris may ulcerate. Most frequently, the lesions appear in the neck and head region, on the nose, earlobes and cheeks. Ulcerative forms of lupus vulgaris are characterized by ulceration that may be severely invasive.

Papulo-necrotic tuberculid. This kind of lesion, symmetrically distributed as reddish papules, is usually located on the extensor regions of the extremities, back, and buttocks, may lead to ulcers formation.

Scrofuloderma. This lesion begins as a bluish-red nodule that may ulcerate. It is located over an infected lymph node or over an infected joint.

Miliary tuberculosis. Lesions of miliary tuberculosis may lead to ulceration, too.

Tuberculous gumma. In this stage of tuberculosis, also called 'metastatic tuberculous abscess', a subcutaneous nodule may lead to ulceration. Lesions may occur in children.

2.4. Vascular lesions

2.4.1. Venous Ulcers

About 70% of leg ulcers are represented by venous lesions in origin. In modern medicine, the prevalence of venous ulcers is quickly becoming lower. This is due to the higher standards of medical care currently practiced. The importance of compression therapy is well recognized nowadays; the use of low-molecular-weight heparins can prevent venous thromboembolism phenomenon in high-risk patients. Furthermore, vein surgery has become truly minimally invasive. Venous insufficiency may be diagnosed in patients already presenting with peripheral arterial disease. In many cases, the direct trigger for ulceration is represented by some external physical injury. Although in a healthy person mild injury does not cause significant damage, in patients with venous insufficiency the skin layer runs a much higher risk of developing this kind of lesions.

Histologically, characteristic microvessels in areas subjected to chronic venous hypertension start to be observed as coiled and dilated; they present a glomerular appearance in intravital capillaroscopy. In the severe disease, the number of functioning and perfused capillaries is dramatically reduced. The severity of cutaneous microangiopathy has been found to directly link to the development of clinical cutaneous trophic modifications. Current studies show that the exact mechanism leading to the histologic evidence of tissue damage in venous insufficiency remains uncertain. Nevertheless, in recent researches it has been acquired an increased understanding of certain physiological mechanisms involved in this process.

For chronic venous insufficiency, the pressure – basically hypertension - in the deep system may be transmitted to the superficial system. Partsch et al. suggested that venous insufficiency is characterized by peaks of pressure occurring with every muscle con- traction and transmitted to the capillary network. It is known that these pressure peaks present a gradual and progressive destructive effect on the capillaries in the skin layer and subcutaneous tissues.

In addition, leakage of fluids from the capillaries to the interstitium of the dermis and subcutaneous tissues directly bring to edema. Whatever the mechanisms directly linked to edema, it has been shown to affect the quality of the skin layer. It leads to sclerotic modifications in subcutaneous tissue, and consequent interference of metabolic and gas exchange. In addition, due to the edema, lymphatic vessels and their valves arrive to

fibrotic changes, with a collateral reduction in normal lymphatic function and drainage of the tissues, which sets up a vicious cycle of edema.

Endothelial damage, therefore, is the result of edema with subsequent impaired oxygenation and interference of metabolic action (and peaks of venous pressure). Intercellular adhesion molecules seem to play a significant role in the pathologic process, as reflected by their expression on endothelial cells. This process is then followed by leukocyte adhesion and the trapping of white cells within the capillaries. Lack of endothelial integrity, in association with the increasing presence of white blood cells, may bring to protracted inflammation and subsequent destruction of adjacent tissue, fibrosis.

In addition, numerous hypotheses have been proposed to explain the mechanism of skin damage and the consequent development of skin lesions in the presence of venous insufficiency.

In 1982 Burnard and Browse proposed that venous hypertension, transmitted to the capillary network, leads to the distention of capillary walls and the consequent widening of capillary pores. Subsequently, fibrinogen molecules leak into the extracellular fluid, giving shape to complexes of fibrin around the capillaries. The pericapillary fibrin layer is ready to form a mechanical barrier, which prevents the transfer of oxygen and nutrients, progressively damaging the skin and subcutaneous tissues.

However, other researchers have proposed that the fibrin cuffs do not exclusively function as a barrier for oxygen transport. If so, these cuffs only

seem to reflect abnormal microcirculation with transmural deposition of plasmatic macromolecules.

Falanga et al. have proposed that growth factors may be trapped by some macromolecules leaking through the capillary pores into the dermis layer. In addition, growth factors seem to be unable to participate and function in the processes of tissue repair.

Since venous pressure and the subsequent destroying effect on tissue is maximal distally, venous ulcers seem to occur in the lower calf. The medial malleolus is more commonly affected than the lateral one. This phenomenon is attributed to the anatomy of the venous system, in which a larger quantity of venous vessels is located medially. Therefore, the medial aspects of the legs are linked to higher venous pressures. In addition, not infrequently these ulcers may appear above the lateral malleolus as well. Lateral venous ulcers usually reflect the presence of an incompetent saphenous vein, in the presence or not of deep venous insufficiency.

2.4.2. Ulcers in presence of peripheral arterial disease

Most patients presenting peripheral arterial disease are over the age of 65. In contrast to the clinic presentation of venous ulcers, arterial ulcers are increasing in number. People get older, and peripheral arterial disease is becoming more prevalent. Arterial ulcers are known to constitute about

12% of leg ulcers. Mixed ulcers, of arterial and venous disease, are considered to affect approximately 12–18% of patients presenting leg ulcers. Arterial ulcers often develop after a physical trauma. The trauma may be minor, but it affects poorly vascularized tissue, which is not able to heal as normally vascularized healthy tissue does. In addition, the trauma site may become the portal of entry for infectious agents, further aggravating the lesion.

Sometimes, arterial ulcers may appear without a previously diagnosed trauma, when critical limb ischemia has developed. Beyond a certain degree of ischemia, there is a complex list of events that may lead to necrosis.

The critical limb ischemia, according to the Trans-Atlantic Inter-Society Consensus Document on the Management of Peripheral Arterial Disease (TASC Working Group), is defined starting from a patient presenting ulcers, chronic ischemic rest pain, or gangrene linked to objectively proven arterial occlusive disease. The suggested inclusion criteria in TASC for the diagnosis critical leg ischemia were absolute ankle pressure below 50–70 mmHg or reduced toe pressure (<30–50 mmHg).

Atherosclerosis of large arteries represents the main process in the pathogenesis of chronic critical limb ischemia. It leads to occlusion or severe narrowing of vessels, with the following reduction of blood flow and decreased perfusion to distal regions. Other parameters such as low blood pressure or the diagnosis of anemia may influence the degree of ischemia.

Since a growing percentage of arterial ulcers are caused by trauma, arterial ulceration may appear anywhere on the lower calves. Ulcers seem to appear in the pretibial or lateral area of the leg or on the dorsum of the foot. They also can appear in the malleolar region. If critical limb ischemia has appeared, it may be clinically diagnoses by distal necrosis of the toes or forefoot. This clinical scenario presents a poor prognosis, and amputation may be mandatory. The dorsum of the feet and heels may also be affected.

2.4.3. Other vascular ulcers

Emboli can cause an acute, rapid development of limb ischemia. An atheromatous plaque that starts to be detached from a blood vessel wall is a large embolus that closes a large vessel and generally affects a specific anatomic area. Cholesterol emboli, in addition, represent microemboli composed of cholesterol crystals (150–250 μm) that can occlude many small arteries with the induction of multiple lesions.

Martorell et al. were the pioneers in the description of hypertensive ulcers, in 1945, represented as ulcers located in the pretibial or lateral area of the leg. These ulcers were known to occur mainly in hypertensive women above the age of 55. Some authors proposed that the Martorell's ulcer is an alternative variant of an arterial leg ulcer. Others are sceptic about the validity of this clinical term, based on nonspecific histologic characteristics

in leg ulcers, clinically diagnosed as *Martorell's ulcers*. However, the elderly population is on a higher risk to develop hypertension, as well as atherosclerotic changes into the blood vessels.

2.5. Oncologic/Immunocompromission, diabetic and pressure sores

2.5.1. Oncologic and immunocompromised patients

For patients presenting immune deficiency states, organisms which usually do not cause ulceration may result in the development of ulcerated lesions: In *HIV-affected patients*, varicella virus infection may lead to ecthymatous varicella zoster. The same patients tend to present a characteristic wound linked to the *Kaposi* onologic syndromic scenario.

Perianal ulcerated lesions have been described for immunocompromised patients due to the herpes virus or CMV.

Ecthyma gangrenosum represents a typical lesion appearing in oncologic and immunocompromised subjects. It begins as a hemorrhagic vesicle or pustule that runs just under the ulceration. In more than 85% of cases, it occurs at the level of the perineal region or on the extremities. Pseudomonas Aeruginosa represents the main cause of these lesions. In addition, Klebsiella and other bacteria have also been isolated. It may occur

in the clinic situation of bacteremia, when it is considered to be a marker of severe prognosis.

Fungal infections are described as more aggressive in immunocompromised patients. Sometimes, atypical fungal ulcerating wounds have been described in patients with unknown immune system disorders.

2.5.2. Diabetic wounds

Peripheral Arterial Disease and Atherosclerosis (Macroangiopathy)

Peripheral vascular disease seems to be more common in people with diabetes than in the rest of the adult Occidental population. In the presence of additional risk factors - such as hyperlipidemia, hypertension, smoking - the incidence is even higher.

The prevalence of peripheral arterial disease in diabetic patients is ranged between 22% and 43%, and it is regarded as a sign of premature damage of blood vessels. A peculiar feature of diabetes is that the ulcers tend to be found more distally than they do in non-diabetic patients affected by peripheral arterial disease.

Diabetic ulcers (due to peripheral arterial disease) could also appear anywhere at the level of the lower calves, usually on dorsum of the foot, on the lateral or pretibial aspect of the leg, or malleolar area. As in peripheral arterial disease, necrosis of a distal toe or foot may evolve if

there is severe ischemia of a diabetic limb. In severe cases, widespread calcification may go on along the length of the media of the arterial wall. Doppler measurement of ankle blood pressure (and consequently *ABI measurement*) may be linked to high pressures, which does not accurately reflect the true degree of ischemia of the limb.

2.5.2.1. Neuropathy

Neuropathy in diabetes hits sensory, motor, and autonomic fibers. It is estimated that almost 25% of type-2 diabetic patients present neuropathy, while it affects 52% of patients over the age of 55 years. Loss of substance of the soles of diabetic patients is, in most cases, directly linked to neuropathy.

The main effects of sensory, motor, and autonomic neuropathy present as the following:

Sensory neuropathy results in anesthesia and loss of protective sensation.

Motor neuropathy is identified as difficulty in activating certain muscle groups, resulting in the lack of distribution of pressure on the sole while walking. Anatomic zones subjected to repetitive focal pressure may ulcerate or, alternatively, may develop a callus, which predisposes to ulceration. The consequences of motor neuropathy end in the presence of typical foot deformities described in diabetic neuropathy, such as protrusion of the metatarsal heads.

Autonomic neuropathy is often linked to dry skin and it contributes to fissuring and callus formation. It also leads to arteriovenous shunting which, although accompanied by increased blood flow, reduces nutritive cutaneous capillary flow.

2.5.2.2. Macroangiopathy - Peripheral Arterial Disease and Atherosclerosis

This is characterized by some particular conditions: *sensory neuropathy* may be linked to symptoms of intermittent claudication and rest pain. An *ischemic foot* may nevertheless present as warm and pink on clinical examination, due to autonomic neuropathy.

The neuropathic process leads to the formation of ulcers on the sole or on the lateral and medial regions of the foot in diabetic patients. Typically, a neuropathic ulcer of the sole is surrounded by limited callus formation. Neuropathy and decreased sensation make the patient even more prone to trauma and following ulceration, which may occur anywhere in the distal anatomic area of the limbs. In some cases, the presence of neuropathy prevents early diagnosis of an ulcer by the affected patient.

2.5.2.3. Microangiopathy in diabetic patients

This condition is characterized by the thickening of basal membranes and increased permeability of capillaries. In its severe stages, it results in compromised gas exchange, a decrease in cutaneous pO2, and ischemia.

The described ischemic changes lead to additional damage to the skin, thereby increasing the probability of a loss of substance. The association of macroangiopathy and microangiopathy seems to be the cause why diabetic ulcerations tend to be located more distally, compared with ulceration in non- diabetic peripheral arterial disease.

Microangiopathic involvement of the *vasa nervosum* results in diabetic neuropathy. The effect of microangiopathy is most well described for the kidneys and the retina. The possible influence of these vascular changes on ulcer formation in the diabetic patient (for the leg) is still under study and has not yet been fully evaluated. It is reasonable to think that they affect capillary function.

Charcot's osteoarthropathy represents a destructive process of the joints, occurring in diabetic neuropathy. It leads to excessive focal pressure on the sole of the foot, predisposing it to ulcer formation. Another process is known as *cheiroarthropathy*, a thickening of the skin with limitation of joint mobility and an abnormal gait, with the subsequent inappropriate weight distribution on the sole of the foot.

Infection is a frequent complication of diabetes, which aggravates tissue damage. Diabetes is associated with decreased phagocytic activity and

decreased function of leukocytes. Chemotaxis of leukocytes and phagocytosis are linked to poorly controlled diabetes. Hyperglycemia has been described as an inhibitor of the cellular transport of vitamin C into fibro- blasts and leukocytes, with reduced chemotaxis of leukocytes.

2.5.2.4. Anatomic location of Ulcers in Diabetes

In view of the above-mentioned pathologic characteristics of diabetes, even minor trauma or otherwise negligible superficial infection may be sufficient to induce ulceration.

In a diabetic patient, ulcers may be located as follows:

- lateral or pretibial regions of the leg, dorsum of the foot, or malleolar regions, due to peripheral arterial disease and subsequent damage to the skin and subcutaneous tissue;
- distal forefoot or distal toes, due to the severe ischemia of peripheral arterial disease; This kind of neuropathy also leads to ulceration mainly on the sole. In addition, the decreased sensation combined with increased susceptibility to trauma can occur anywhere on the distal limb. Osteoarthropathy also contributes to the formation of plantar ulcers.
- The typical diabetic ulcer is often located on the sole. Due to the combination of several detrimental factors including neuropathy, macro-angiopathy, microangiopathy, and reduced resistance to

infections, ulcers in diabetes can, in fact, occur anywhere on the lower leg.

2.5.3. Pressure Ulcers

Pressure ulcers are described in literature as a model of continuous injury. The prevalence of pressure ulcers widely ranges from population to population. Elderly patients, or patients who are hospitalized or bound to a wheelchair, are particularly prone to pressure ulcers. They may occur in an immobilized or bed-ridden patient of any age. Among patients admitted to hospital following general surgery interventions, the prevalence, from one to five years after the initial injury, is between 24% and 38%.

Two other widely accepted terms for pressure ulcers are *decubitus ulcers* and *pressure sores*.

Local pressure on the affected site is the most important etiologic factor, with the degree of pressure and its duration being the most significant components. Pressure ulcers present as developing in areas where soft tissues are compressed for prolonged periods between a bony prominence and any external object, but they may also evolve over any cutaneous area that is exposed to continuous pressure. Pressures exceeding standard capillary pressure lead to a reduced oxygenation and impair the microcirculation of the affected tissue. Garfin et al. presented a work that stated the value of 30 mmHg as the critical limit for ischemia. However,

high pressures of 70 mmHg or more, which may occur when a patient lies on a standard hospital mattress, may lead to tissue death within a few hours. Most of the potential damage may be prevented by relieving the pressure intermittently. Therefore, prolonged immobilization represents a significant risk factor. This factor emphasizes the importance of frequently moving (every two hours minimum) and repositioning immobilized patients in order to prevent the formation of pressure ulcers.

Apart from pressure, there are other mechanical factors like shearing forces and friction which may enhance ulceration. Significant topical factors include excessive moisture and exposure to chemical irritant substances. Both the general condition and nutritional situation of the patient play a major role in the origin of pressure ulcers.

More than 91% of pressure ulcers appear on the lower regions of the body – 67% in the pelvic region and 38% on the lower legs. Heels and malleoli are also frequently affected.

The clinical appearance of a pressure ulcer depends on its severity. Many gradation scales have been proposed. A commonly accepted system was presented in the USA in 1987 by The National Pressure Ulcer Advisory Panel (NPUAP).

Pressure lesions are characterized by peripheral undermining. At any stage, the initial impression of the depth of a pressure ulcer may be misleading and the ulceration of the tissue may be much deeper and more extensive than originally diagnosed.

CHAPTER 3

Diagnostic and therapeutic tools, from translational research to clinical practice

3.1. Skin lesions and clinic evaluation of the surgical patient

The skin represents the most extended organ of the human body. Throughout the lifespan a person's skin is exposed to a large number of potential damages. In a healthy person the skin is compact, strong, and has the property to repair itself in response to all but the most severe of insults. However, human skin may be subject to changes that result in it becoming dysfunctional, impaired and vulnerable.

3.1.1. Patient history

One of the most important factors of assessing the skin of the surgical patient is to ensure that a detailed history is taken focusing on the most important aspects of the patient's story. A good anamnesis should receive the most time during the first consultation and the attention allocated to undertake this essential aspect of the discussion should reflect this. A comprehensive assessment is absolutely required. Focusing on the

diagnosis of the underlying cause of a wound is essential. The cause of the wound must be found and identified; if it is a chronic wound then the underlying factors that may have contributed to or are contributing to the chronicity should be found so that they can be avoided. If the patient is not able to provide the requested then secondary sources of data will be required. It is also important to determine what medications patients are taking and why they are taking it. It's fundamental to establish if the patient presents any allergies; Surgeon should obtain a detailed description of the allergic response to medication or any allergens, for example specific dressings, latex gloves. Any allergies must be clearly recorded in the person's notes. The association between cigarette smoking and delayed wound healing is well recognized in clinical practice. Alcohol consumption, at the same time, can increase the risk of infection and interfere with wound closure. Estimating alcohol consumption can help determine if intake is a potential risk factor.

3.1.2. Physical assessment of the skin

Examination of the skin must go hand in hand with the taking of a detailed history. It's important to visit the patient under a good light when examining the skin; there are a number of reasons why an assessment or examination of the skin is needed. A number of disease states can be

shown on the skin. Findings can represent a disease process that is limited exclusively to the skin or there may be evidence of systematic disease.

Full examination (inspection, palpation, percussion, auscultation) should include all body surfaces and examination of the skin layer, nails and hair should take place as well as the mucous membranes. An entire body examination is not needed if there is a quickly recognizable lesion (a localized process). A systematic approach to skin assessment should be adopted and a head-to-toe approach is recommended. A body map should be used to document findings. There are a variety of tools that can be used to undertake an assessment of the skin. Common tools in use are predictors of risk and are related to the assessment and prevention of pressure ulcers. A combination of intrinsic and extrinsic factors can result in the formation of a pressure ulcer.

The Waterlow Pressure Ulcer Risk Assessment/Prevention Policy Tool is the most commonly used assessment tool, it is also the most easily understood.

Many surgeons make the common mistake of looking at and noting the condition of the wound without first considering the patient as a whole. They apply dressing or treatments to a wound that may be contra-indicated for a medical condition the patient may have. For example, they may regularly change the dressing type because the wound is not progressing as it should, which could be that the patient is not eating sufficient amounts of proteins to promote healing.

3.1.3. The inverted pyramid approach to wound assessment

This kind of process represents a 360° ongoing cycle of assessment that continually focuses on anything that could be delaying the wounds progression to healing. It is a process that when documented, provides

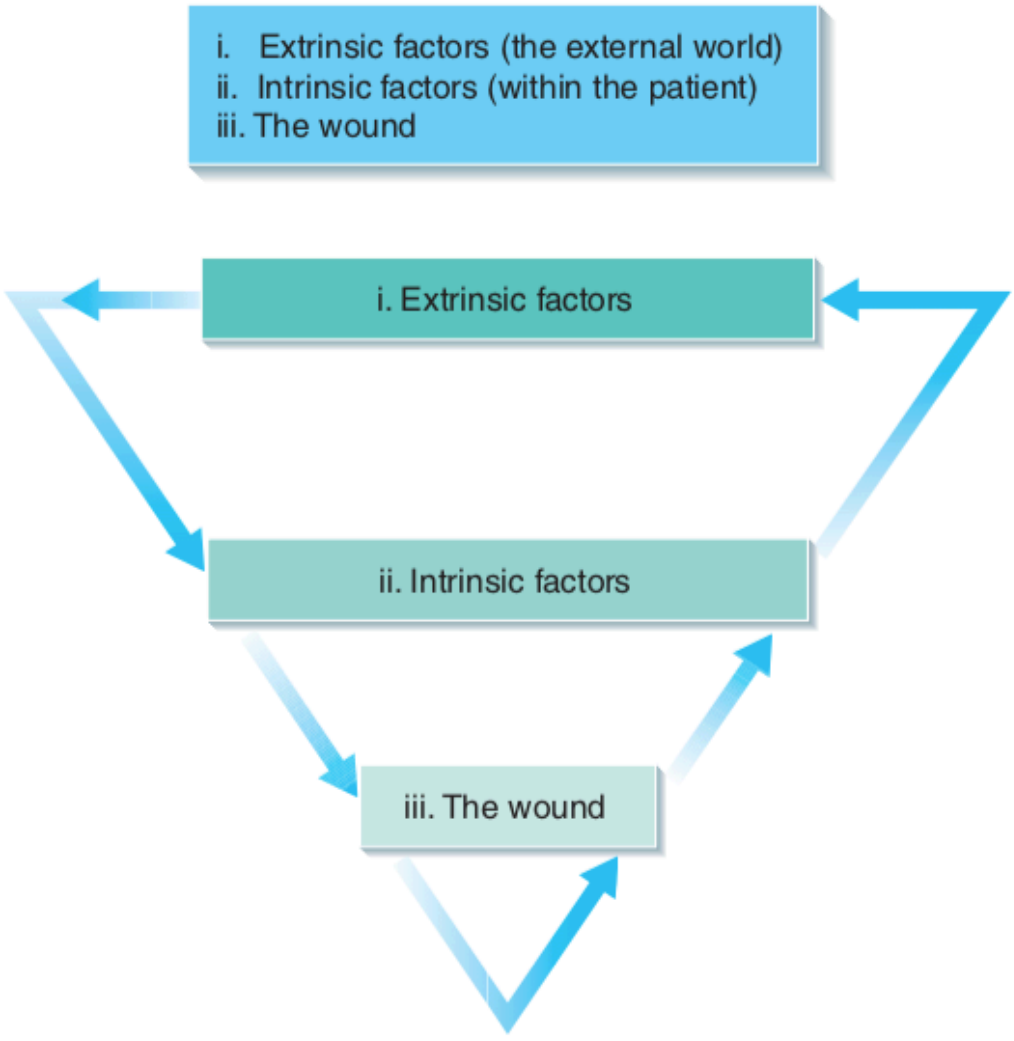

Figure 3.1. The inverted pyramid approach to wound assessment.

evidence to the multidisciplinary group of physicians about the wounds progress and any factors that could be hindering that process.

Many extrinsic factors that prevent a wound from healing can be improved upon once it has been identified and given due consideration as part of this process. The patient whose wound is not improving because inadequate pressure relieve is not being provided will present improved healing rates once frequent position changes are introduced; in other words, the cause of the wound has been identified on assessment and adequately addressed (as far as possible) in order to improve healing rates. Next it is important to consider any underlying medical conditions the patient may have and as far as the surgeon can deal with these; for example, the patient may be a diabetic and it is crucial that blood sugar levels are monitored and maintained within acceptable levels in order to improve wound healing. Many intrinsic factors are unchangeable, such as age. However, others can be improved upon such as quality of nutrition.

3.1.4. Wound assessment

The surgical wound assessment includes the anatomical site of the wound, the dimensions and depth of the wound, the tissue type on the wound (the state of granulation tissues, slough, necrosis); the condition of the surrounding skin; levels and type of exudate oozing from the wound; signs of maceration or excoriation; signs of infection and pain levels, including aggravating factors for pain. Reassessments of the wound, along with the extrinsic and intrinsic assessments ought to be carried out at intervals

determined appropriate for that wound type. A wound should be reassessed (using the above process) once a week in the hospital and monthly as a minimum after the hospitalization. When a wound presents as infected, more frequency assessments will be required, in order to monitor for potential systemic infection and eventually change the antibiotic therapy.

3.2. The Five Steps to Evidence-Based Clinical Practice and how the Translational Research has changed the therapies.

3.2.1. Evidence-based wound care: the five steps for a good practice, from the translational research to the clinical practice

Evidence-based wound management represents a combination of best translational research evidence with clinical expertise and patient values.
Wound care has often been taught through case examples and what was considered best clinical practice.
With the increased importance of providing an evidence base to practice, there is now a need and a requirement to move away from indiscriminate experimentation, clinical practice associated with outmoded, unjustified opinions, to learning that has an evidence base. The evidence available ranges from expert opinion to randomized clinical trials.

Healthcare practitioners must strive to provide the safest and best quality care they can. Evidence-based wound care is required because of the increasingly complex nature of health care and health care decisions.

This process should be ideally broken down into a number of stages. These are five stages associated with evidence-based practice. 1) The first step is recognizing that *there is a need for new information*. Precise answers can only be provided when a precise question has been posed. Failure to ask a focused and precise clinical question can be a major threat to evidence-based practice. 2) *Choosing the right evidence* is of central importance. There are several sources of evidence, and it can be hard to know where to begin. The main sources of evidence often come from more experienced colleagues or textbooks, however there are problems with these sources of information. When using evidence from textbooks the opinions expressed may be out of date before the book is even published, or incompatible with current best evidence. A number of groups have established levels or hierarchies of evidence, usually based upon scientific merit in an empirical model. When examining the evidence it will be helpful to consider the hierarchy of evidence. 3) *The evidence must be critically appraised* to establish its validity and potential usefulness. The main issues are about the possibility to trust the evidence, its mean and its everyday practice. 4) Once it has been established that the evidence is of quality, another decision will need to be made whether the evidence should be incorporated into the everyday clinical practice. Consideration of both the benefits and risks of implementing the change, as well as the benefits and

risks of excluding any alternatives should be undertaken. These decisions should be made in collaboration with the multidisciplinary team, managers and patients where appropriate. Resistance to change should be given serious consideration, as this can be a challenge. 5) Evaluation and reflection are fundamental to understand whether the actions taken have achieved the desired results. This is a basic aspect of health care practice. Evidence-based practice represents a continuous and cyclical process, especially for the translational research. Evidence-based wound care, in particular, should focus on employing a questioning approach to the everyday practice.

3.2.2. How the Translational Medicine and Surgery changed the current standard of therapies

Wound healing represents a complex biologic system depending on the timed coordination of several cell types, intra and extracellular mechanisms, molecules, and pathways, but also on extrinsic factors like infections or mechanical irritation. Every defect, loss, or dominance of one factor of this convoluted interaction can cause a breakdown of the global system, resulting in chronic wounds and a dramatic loss of quality of life.
However, no drug can be effective when its sustained and targeted delivery to the wound site cannot be identified. Specific *drug delivery systems* (*DDSs*) are key for achieving this goal. An efficacious DDS addresses the obstacles presented by the wound environment and prevents the wound

from oxidative, mechanical and enzymatic stress and from bacterial contamination; it also provides enough oxygen while stabilizing localized and sustained drug delivery to the target tissue.

The importance of drug delivery and the challenge for translational medicine and surgery to promote effective DDS for wound healing applications are represented by the large number of recent studies regarding this topic. Different routes of drug application warrant strategies to face the obstacles represented by the systemic circulation and the harsh wound environment.

3.2.2.1. Biological therapies: bioactive scaffolds, Growth Factors, NPWT, Stem Cells and RNAi

Studied for their ability to treat acute and chronic wounds, biological therapies functionally aim to restore the body's natural regenerative capabilities. Creating microenvironments that promote proliferation of both matrix depositing stromal cells and endothelial cells at the site of injury, biological therapies may make easier the formation of a vascular network in newly forming tissue. Biologic approaches include *bioactive scaffolds, growth factor-based therapies, and stem cell-based therapies.* Acellular scaffolds have the objective to provide coverage to the wound site, creating a matrix for resident cells infiltration, and promoting granulation tissue formation. Despite these promising findings, a general uncertainty regarding the best source of tissue and processing technique

guide surgeons to choose products based on cost, familiarity, and availability, rather than efficacy. The lack of randomized controlled, head-to-head trials between products, and studies often sponsored by the commercial manufactures themselves, increase the risk of potential bias. In addition, a constant fear surrounding the use of these devices is represented by the risk of disease transmission and donor rejection of the graft. Because wounds vary in vascularity, the presence of infection, and quantity of debris, it is essential for surgeons to prepare the wound site in order to allow these scaffolds to become successful. Growth factor therapies are based on an understanding that specific regulatory pathways rule the host response to wound healing and are used to stimulate re-epithelialization, wound angiogenesis and matrix deposition.

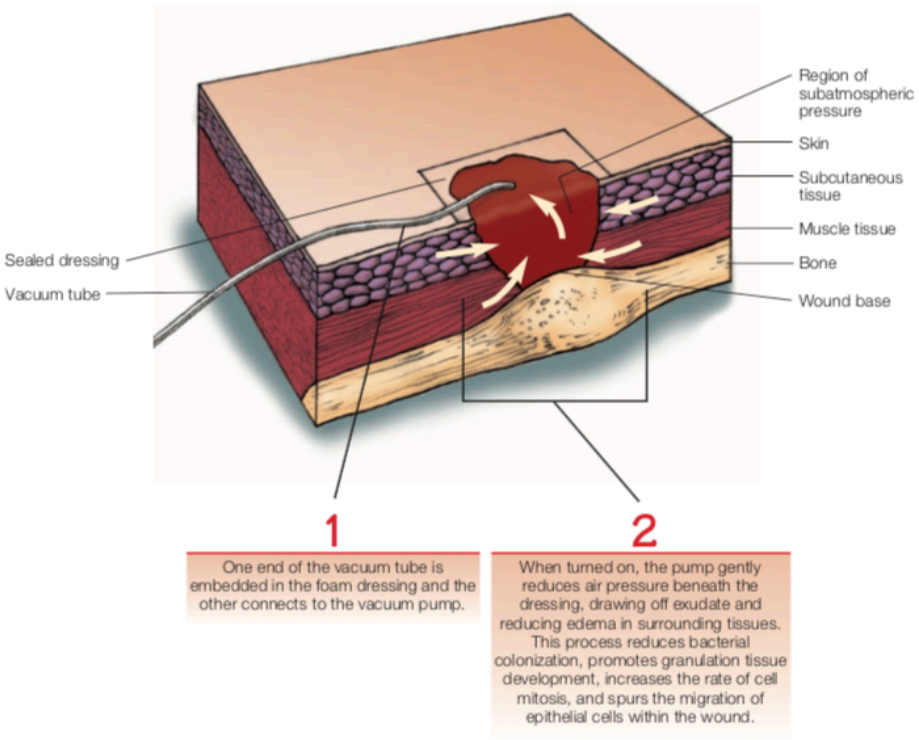

Figure 3.2. NWPT: principles.

Negative-pressure wound therapies (*NWPT* also known as vacuum-assisted closure, VAC therapy) provide an alternative way of increasing effectors of wound healing and neovascularization locally. NWPT temporarily leads to relative hypoxia in the wound region resulting in significant higher levels of local regenerative factors such as VEGF, FGF β, TGF, angiopoietin 1, and BMP 2, and its application has shown benefits regarding bacterial contamination. In addition, several studies suggest that the micro-deformation of the wound surface is linked to accelerated cell migration and matrix production. By using silver-coated foams, the NWPT can be even more effective in preventing or treating bacterial contamination. *Stem cells* are characterized by their ability for self-renewal and the capacity to differentiate into various tissue types through asymmetric replication. The trophic activity of these cells is linked to the development of cell-based approaches for the treatment of chronic wounds. Growth

factors released from stem cells stimulate local cell migration and proliferation, increased angiogenesis, and antimicrobial activity.

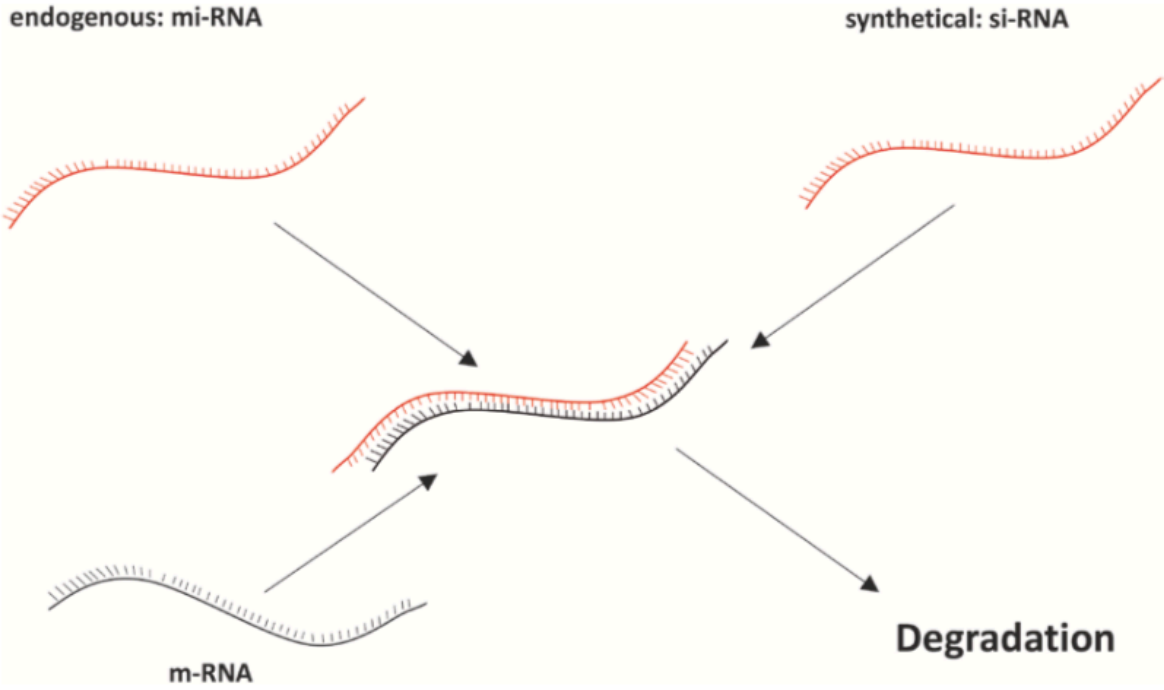

Figure 3.3. Principle of RNA degradation.

Far from traditional pharmaceutical approaches, the silencing of gene function through *RNAi* leads to selective targeting of molecules that have been hard to regulate using growth factors and small molecule-based therapies. RNAi represents a powerful gene-silencing system with a lot of potential for therapeutic application in wound care. Inhibiting gene expression at the post transcriptional level, RNAi (micro-RNAs and small interfering RNAs) targets specific mRNA molecules for destruction and offers an unexplored therapeutic approach to wound healing. Despite broad therapeutic potential, the effective delivery of RNAi to target cells in vivo has still a significant challenge due to the high rates of degradation by

ubiquitous RNases, the targeting of specific tissues, and the maintenance of long-term silencing. Developing a controlled DDS for RNAi is fundamental to make real the full potential of these next generation therapeutics.

3.3. Wound care and TIME

The English acronym T.I.M.E (Tissue, Infection or Inflammation, Moisture imbalance, Epidermal margin) was created to better frame the principles of chronic wound bed preparation ("Wound Bed Preparation"). Through TIME it is possible to carry out a systematic review of all the objectivable characteristics of the lesion, easily identifying the major pathological elements and the most appropriate interventions to remove all those barriers that prevent healing.

3.3.1. Tissue (necrotic or devitalized tissue)

The presence of non-viable tissue (necrotic tissue, eschar, fibrin deposits) prevents healing and evaluation of the size and depth of the lesion; moreover, as already mentioned, it favors infections. It is therefore through debridement that the removal of the necrotic / devitalized tissue

is obtained to restore the vitality of the bottom of the lesion and in particular of the extracellular matrix. Debridement is divided into:

• surgical, first choice if the lesion is extensive, but requires experience of the procedure and caution in immunocompromised patients and on anticoagulant therapy;

• enzymatic, slower than the surgical one, which is implemented through the use of collagenase, a product that better concentrates efficacy, tolerability, ease of administration and selective action on non-viable tissue, saving the healthy one;

• autolytic, through dressings that create a moist environment favoring the action of phagocytes, cleansing and the formation of granulation tissue;

• mechanical, suitable for necrotic wounds in the inflammatory phase, but to be avoided in the presence of wounds in the granulation phase.

3.3.2. Infection/Inflammation

In this case it is essential to observe if there are local signs such as change in color of the wound bed, erythema (> 1-2 cm), soft tissue edema, worsening of pain, exudate serous or purulent, the presence of biofilm, bad odor, bright red friable granulation tissue and fistulas.

Local signs must also detect the presence of fever, vital parameters, laboratory markers of infection and optimally treat the concomitant causes that compromise the host's defenses.

Wound culture examination is essential to detect bacterial load and to optimize the use of targeted antibiotic therapy in addition to adequate wound dressing that includes cleansing, debridement and antisepsis.

3.3.3. Moisture Imbalance (maceration or dryness)

In this case we can observe two opposite situations:

- dryness, which is associated with a slow migration of epithelial cells;
- excess exudate with maceration of the wound margins.

The right intervention in these cases consists in the application of advanced dressings to promote the right degree of humidity or a compression bandage, negative pressure or other methods to remove excess exudate.

3.3.4. Epidermal Margin (margins that do not progress on the wound bed)

In this case, at the level of the wound, there is a lack of progression of the margins in the absence of necrosis, infection and exudate. Epithelial margins may appear hypertrophic or undermined or with altered granulation tissue. Concomitant is the persistence of pain associated with the wound.

In this case, a new classification of the wound and of the patient is necessary, perhaps investigating the presence of concomitant pathological factors that have arisen de novo or that, even if known, are in a phase of clinical decompensation (vasculopathy, edema, diabetes mellitus, malnutrition, alcoholism, previous surgery or radiotherapy, congenital neutropenias, use of steroid drugs, immunosuppressants or non-steroidal anti-inflammatory drugs).

If with a new reassessment of the wound status a well-prepared wound bed is obtained, but which still fails to heal, innovative therapies are needed that trigger the healing process including autologous skin grafts, transplants of cultured cells / keratinocytes, bioengineered products, allogeneic tissue, artificial skin, stem or bone marrow-derived cells, use of growth factors (bFGF, TGF-β, EGF, PDGF). It should be remembered that, however, the candidate patient for these techniques is the one who has a well- prepared wound bed, on which these therapies are effective.

3.4. Experimental clinical application of a virtual measurement tool, imitoMeasure ©

3.4.1. Background

Lesions of skin and soft tissues are widely included among the most frequent wound types diagnosed in the hospital environment. Assessment of wounds morphology represents, in the everyday medical activity, the first step for the correct pathway of diagnosis. In this perspective, the traditional measurement methods (paper rulers) are about to be enriched by the newest ways to use technology in the everyday clinical practice, which represent the future of wound care. Costs and subjective variations can be considered as the main obstacles these devices must face. The ideal method should be unexpensive, versatile and easily reproducible. The advent of last generation smartphones, equipped with high resolution cameras, gives the chance to look for this solution into a downloadable app. Authors present a pilot study focused on the statistical analysis of 32 cases of wounds measurements conducted by both the traditional method (paper ruler) both the digital smartphone analysis.

3.4.2. Patients and Methods

This study was conducted at the Surgical Division and at the local referral center of wound Care of Parma Hospital (Parma, Italy). This work was approved by the local Ethics Committee of Emilia Romagna (AVEN) and all the patients gave their informed consent before the enrollment. Authors included patients available to undergo subsequent follow-up of the study and capable of providing informed consent. On the other side, patients not available to undergo subsequent follow-up of the study and unable to provide informed consent were excluded. 32 lesions were morphologically analyzed. All the enrolled patients were evaluated by both the traditional method (paper ruler) both a digital smartphone analysis based on the app imitoMeasure (owned by imito AG, all rights reserved). The app was tested on Apple iPhone X device running iOS 13 and it includes functions for photography, documentation, capture and measure of cutaneous wounds. All the pictures were taken from a 25 cm distance by the same device and all the collected data were encrypted. A parallel traditional measurement with a paper ruler was taken for every lesion, too. In order to obtain digital measurements, a calibration marker (2 cm diameter) was positioned next to the wound prior to take the picture. This adhesive is used by the system to calibrate colors, distances and spaces, so that the width, the length and the area of the lesion can be calculated. The extracted data were compared to the traditional measurements and a statistical analysis was based on intraclass correlation coefficients (ICC) calculated using a PC running SPSS

version 18. Internal reliability was considered as “excellent” for ICCs = 0.90-1.00.

3.4.3. Results

32 patients, aged between 66 and 87 years (median age: 76,2), were enrolled for this pilot study over a one-year period (from March 2019 to February 2020). The evaluated lesion types (one for each patient) were classified as follows: 14 surgical site lesions - (44 %); 9 infectious or vascular etiology (SSTIs) – (28%); - 9 presented ulcers from mixed etiology (28%). Three morphological parameters were evaluated: width (expressed in cm), length (expressed in cm) and area (expressed in cm2). Each measurement was taken three times at a distance of 25 cm and the statistical analysis allowed authors to calculate intraclass correlation coefficients (ICC). Considering the width and the length, the digital measurements resulted really close (in the most of cases identical) to the traditional system (ICC = 0,99 for each) (Table 3.1). The area (expressed in cm2) was found to be the less comparable (ICC = 0.95) (Table 3.2), but the data were close in this case, too.

3.4.4. How the technology can change the wound assessment

Wounds morphological assessment represents a fundamental preliminary phase in everyday clinical practice. Its imprecise execution can potentially lead to suboptimal wound care, delayed healing, increased risk of infection and major economic impact, too, as underlined by Wang et al. During the preliminary wound assessment, dimensional parameters (such as width, length, depth) are described as well as the general condition of the skin, the chromatic parameters and the global appearance of the lesion.

The present hi-tech era gives the great opportunity to obtain objective and reproducible measurements, as described by Khong et al. Although the paper ruler represents the cheapest method, modern technology offers new strategies in this field, as well as the digital wound photography. In this scenario, the global spread of smartphones and apps has increased the options. The traditional measurements supported by a paper ruler can bring precise data on length and width, but much less on the area, due to the morphologic irregularities that can be found in clinical practice. For this reason, traditional measurements can be easily considered less reliable when applied on irregular lesions.

Authors realized a pilot study focused on the statistical analysis of 32 cases of wounds measurements conducted by both the traditional method (paper ruler) both the digital smartphone analysis (with the app imitoMeasure). Three were the analyzed parameters: width (expressed in cm), length (expressed in cm) and area (expressed in cm2). For each

parameter, the statistical analysis showed that digital technology became highly reliable. The collected data, secondly, can be easily catalogued and reproduced. Authors must also focus on some limitations of this study, starting from the small number of enrolled patients and the relatively limited number of large lesions: this could have led to a lower percentage of discrepancies among the data. The digital measurement should be tested on several and different devices, in order to be considered homogeneous. Future studies should also define exclusion criteria for wound parameters that could influence the results, as well as the anatomic region. These characteristics can be considered the first step to increase the number of enrolled patients, for future evaluations.

	FIRST MEASUREMENT	SECOND MEASUREMENT	THIRD MEASUREMENT	TOTAL
Width (cm)	1.00	1.00	0.99	0.99
Length (cm)	0.98	1.00	1.00	0.99
Area (cm^2)	0.93	0.95	0.96	0.95

Table 3.1. Comparison between digital and traditional systems: ICC data for each measurement (32 total lesions).

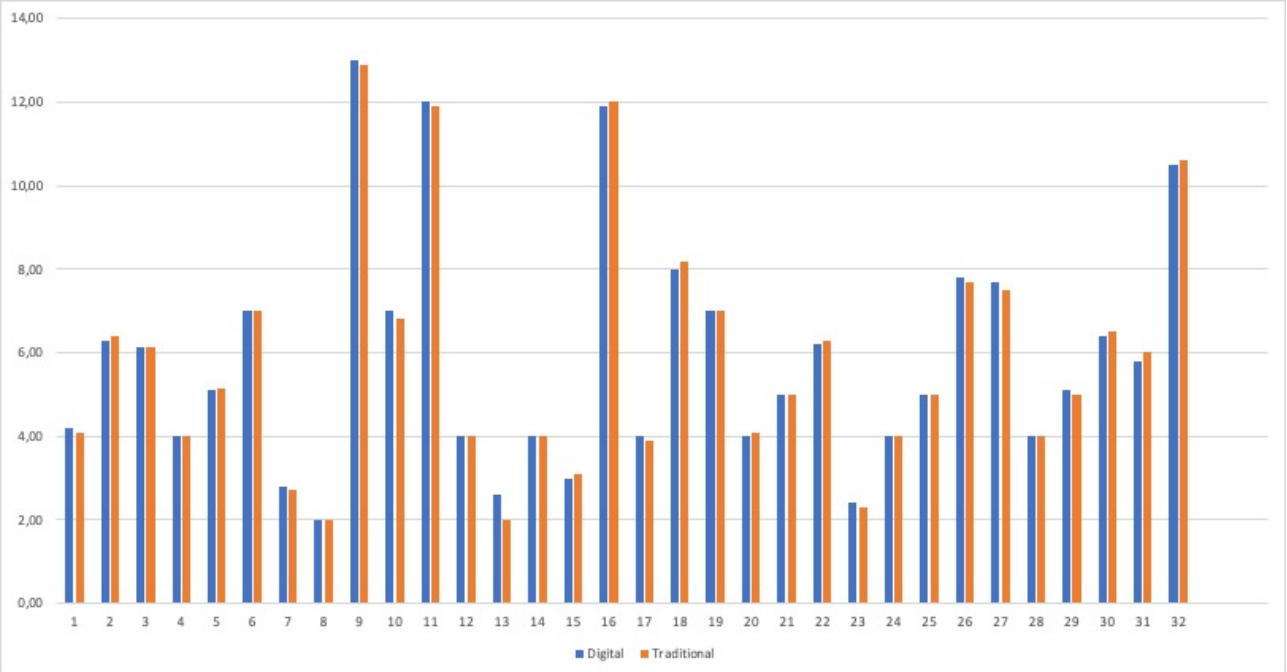

Table 3.2. Wound areas, digital and traditional measurements (three measurements).

CHAPTER 4

The modified TIME-H scoring system, a versatile tool in wound management practice

4.1. Background

Ulcers of skin and soft tissues, in particular those characterized by an infectious etiology (SSTIs), and those connected to the surgical site (SSI), are the most frequent surgical complication in the hospital environment. Many works and meta-analyzes have tried to focus on this clinical problem: it's estimated that, approximately, a percentage of surgical patients between 2.7% and 7.1%, develop SSTIs. Modern technological innovation has led to new and powerful pathways in the treatment of skin ulcers with an infectious origin. Despite the introduction of new therapeutic strategies based on antibiotics, advanced dressings and less invasive surgery, the diffusion of a simple decision-making algorithm is still useful to guide the physician from the preparation of the wound bed to the complete healing. The clinic evaluation of the lesion and the global health conditions of the patient represent the first step in establishing a correct healing prognosis. The general concept of WBP (wound bed preparation) has dramatically changed the way to diagnose and correctly identify the best therapy about the widespread clinical problem of skin lesions. It is exactly from this point of view that the TIME protocol was described and introduced, which stands

for “Tissue, Inflammation - Infection, Moisture, Edge - Epithelialisation”, with the objective of promoting the acceleration of the wound repair process. As largely debated in literature, the TIME protocol presented some limitations in its field of application, assumed that it was unable to give a fundamental answer to patients suffering from chronic skin lesions: the correct quantification of the prognosis in terms of healing time. The TIME-H scoring system was then proposed, which included a score based on the patient’s general health and topical skin conditions. A healing score was thus calculated, which indicated the estimated time of wound closure, in order to obtain the elaboration of a personalized therapy protocol. The system involved assigning a numerical value to each parameter, and it has been modified several times in the literature, as proposed. Just starting from the modified TIME-H this work describes the preliminarly conducted study, with the aim of assessing the impact of skin lesions and soft tissues for the surgical patient.

4.2. Materials and Methods

This study was conducted at the Surgical Division and at the local referral Center of Wound Care of Parma Hospital (Parma, Italy). Starting from the modified TIME-H score, 38 patients were preliminarily evaluated. The patients were classified according to the lesion examined, in particular among those who had an infectious or vascular etiology (SSTIs), and

patients with surgical site lesions (SSI) and assigned to one of three categories prognosis: favorable (with healing expected within 12 weeks) (0-3A, 0-1B), intermediate (with healing expected over 12 weeks) (4-6A, 2-4B) and uncertain healing (7- 8A, 5-8B). This work was approved by the local Ethics Committee of Emilia Romagna (AVEN) and all the patients gave their informed consent before the enrollment. Authors included patients with at least one chronic lesion (present ≥ 3 months) or a dehiscence of a surgical wound that appeared in the immediate post-operative period (21 days), patients available to undergo subsequent follow-up of the study and patients capable of providing informed consent. Authors excluded from the study patients undergoing surgical revision of the lesion; patients not available to undergo subsequent follow-up of the study and patients unable to provide informed consent. Once assigned a score to a patient, the expected result was documented in a database. The patients were then treated with the help of a therapeutic protocol based on an appropriate standard for etiology and wound conditions, choosing in this phase between traditional dressings and advanced dressings (according to the international, national and Emilia- Romagna Region guidelines in both cases). In each subsequent follow-up the same lesion was re-evaluated and the TIME-H score, based on the state of the current wound, was then updated. For the purpose of this study, patient follow-up was continued until complete healing of the wound or the end of the study period, depending on which event occurs first. Authors also collected other informations for each patient, always based on TIME-H score, including

percentage of healed wounds, duration (expressed in terms of weeks) of wound healing, duration (expressed in terms of weeks) of the hospital stay and the subsequent one outpatient evaluation, the rate of change in the size of the wound (cm2/ month) and the final outcome of the healing process at the end of study period. The results of the medians are then put to comparison based on the different categories of lesions and therapeutic strategies. The Mann-Whitney U test allowed authors to analyze data (expressed as + standard deviation [SD]-mean) and to compare values. All changes with a P value of .05 or less were considered statistically significant for the study.

4.3. Results

38 patients were enrolled for this study over a one-year period (from March 2019 to February 2020) (Table 4.1).

Of these 7 were excluded (they were lost during the follow-up). Of the remaining 31 patients, 16 (52%) were male aged between 67 and 86 years old (median age was 77). 15 patients (48%) were female, aged between 64 and 88 years old (median age was 76,7). The evaluated injury types were classified as fol- lows: 13 surgical site lesions - (40 %); 9 infectious or vascular etiology (SSTIs) – (30%); - 9 presented ulcers from mixed etiology (30%). Studied subjects reported their chronic lesions to have been present for a median of 6 months before the first evaluation. The modified TIME-H

score questionnaire also allowed to calcu- late the median wound size (6,8 cm2), with a total median score of 4.0 (range 3.0 – 5.0). After the first evaluation, 6 (19,35%) patients were classified in the certain healing category, 16 patients (51,60%) in the uncertain healing category, and 9 patients (29.05%) in the difficult healing category. A total of 5 patients of the six in the certain healing category presented effective total healing; 12 of 16 patients in the uncertain healing category and 4 of the 9 in the difficult healing category have been correctly classified according to the original prognosis. At the end of the one-year observation period, authors established the healing prediction rate among the studied lesions: the surgical site lesions presented the highest percentage of predictivity (88%), followed by the mixed etiology (72%) and the infectious/vascular injuries (63%). Authors also evaluated the duration of specialist intervention and the reduction in wound size for the three categories (Table 4.2.).

4.4. How the Research faces the problem of the prognosis for the surgical patient presenting chronic wounds

The management of chronic lesions of the skin and soft tissues, especially those with an infectious etiology (SSTIs), and those charged to the surgical site (SSI), represent an important postoperative challenge in the hospital environment. If primary intention closure is not suitable, or if part of a wound closed by this method requires secondary intention closure, the most important goal is to select the most appropriate treatment for the

wound. The available treatment options will depend on the findings on wound and global patient assessment and on the local situation of the wound at the given time. Surgical, sharp and autolytic debridement represent several ways to remove dead and devitalised tissue on the wound bed (e.g. necrosis, gangrene, slough) or infected tissues. Topical negative pressure is a method of wound healing that can only be used once the wound is free from dead and devitalised tissues. Negative pressure is applied to the wound bed, which then promotes an increase in the blood supply to the wound bed. This increases the rate of angiogenesis and therefore the growth of granulation tissue. It removes excess exudate, therefore maintaining a moist wound healing environment. As it removes the exudate it maintains minimal levels of bacteria on the wound bed, thereby reducing the risk of wound infection whilst it is in operation. Healing rates with this method are usually quicker than with traditional methods of healing. Topical negative pressure is also known as vacuum-assisted closure (VAC). TIME (acronym for Tissue, Inflammation / Infection, Moisture, Edge / Epithelialisation) repre- sents a protocol developed on the basis of the “wound bed preparation concept” (WBP), in order to promote an acceleration of the healing process. The modified TIME-H version has been later developed, with the addition of a healing score (H) based on the wound conditions, the systemic state of the patients and the associated chronic pathologies. Authors started from the modified TIME-H proposed by Lim et Al., in order to clearly quantify the prognosis of chronic wounds and improve patients’ satisfaction. This study was conducted at the

Surgical Division and at the local referral center of wound Care of Parma Hospital (Parma, Italy). Authors have studied the 38 enrolled patients prospectively, and involved individuals in different levels of health in determining the Modified TIME-H score for chronic lesions. Authors discovered that scoring lesions through the Modified TIME-H system, a higher proportion of patients in the certain healing category can be predicted to achieve complete healing, with a higher rate of wound size reduction, and a shorter duration of clinical follow up, when compared with other categories of predicted outcomes. This modified TIME-H scoring system should be considered as a ready-to-use daily assessment tool, easily applicable even when the prognosis of patients is not favorable. This is the first report that discovers the several healing predictivity rates among several wound types. At the end of the one-year observation the surgical site lesions presented the highest percentage of predictivity, followed by the mixed etiology lesions and the infectious/vascular injuries.

The limitations of this study are represented by the relatively small number of enrolled patients and short duration in follow-up. Authors simply suggest additional studies, involving multiple centers, with a larger population and longer follow-up to better confirm the validity of this Modified TIME-H scoring system.

This preliminary report showed that this modified TIME-H score should be addressed as a versatile and useful scoring tool that should be used in daily clinical practice for the study and treatment of chronic wound diseases. The current standards of a correct clinical practice cannot ignore the

growing economic and social impact of chronic wounds, reason why the reduction of the treatment period represents a precious target: authors found that by applying the described method, the average healing time was considerably reduced.

Wound score	0	1	2
Tissue necrosis (%)	0	<50	≥50
Infection	Contamination	Colonisation	Infection
Moisture	No exudate	Exudate	Smelly exudate
Epidermal reconstruction (%)	>90	90–30	<30
Wound score	**A**		**B**
Age (years)	≤ 70		>70
Mental state	Good		Poor
Self-sufficiency	Good		Very poor
Nutrition	Good		Poor
Predisposing disease	Absent		Present
Predicted outcome categories	**Score**		
Certain healing (within 12 weeks)	0–3A, 0–1B		
Uncertain healing (more than 12 weeks)	4–6A, 2–4B		
Difficult healing (healing unlikely)	7–8A, 5–8B		

Table 4.1. Modified TIME-H scoring system.

Modified TIME-H category	**Number of patients**		**Duration of specialist intervention**		**Reduction in wound size**	
	Healed % (n)	***Unhealed % (n)***	***Healed months median (IQR)***	***Unhealed months median (IQR)***	***Healed (cm2/ month)***	***Unhealed (cm2/ month)***
Certain healing	83,33 (5)	16,67 (1)	2.6 (1.9–3.3)	1.6 (1.5–1.7)	8.9 (2.6–4.4)	1.2 (1.1–1.3)
Uncertain healing	75 (12)	25 (16)	2.7 (1.3–3.9)	4.2 (3.3–5.5)	0.94 (4.70–0.55)	0.22 (–0.83–1.56)
Difficult healing	44,44 (4)	55,56 (9)	2.5 (2.6–3.7)	2.7 (2.2–3.7)	0.55 (2.2–0.68)	–1.8 (–5.2–1.3)

Table 4.2. Results of the study.

BIBLIOGRAPHY

- Adeyemi A, Waycaster C. Cost-minimization Analysis of Negative Pressure Wound Therapy in Long-term Care Facilities. Wounds. 2018 Feb;30(2):E13-E15.

- Ahmad Fauzi MF, Khansa I, Catignani K et al. Computerized segmentation and measurement of chronic wound images.Comput Biol Med 2015; 60:74–85.

- Aljghami ME, Saboor S, Amini-Nik S. Emerging Innovative Wound Dressings. Ann Biomed Eng. 2019 Mar;47(3):659-675.

- Azuma K, Izumi R, Osaki T, Ifuku S, Morimoto M, Saimoto H, Minami S, Okamoto Y (2015) Chitin, chitosan, and its derivatives for wound healing: old and new materials. J Funct Biomater 6:104–142.

- Badylak SF (2002) The extracellular matrix as a scaffold for tissue reconstruction. Semin Cell Dev Biol 13(5):377–383.

- Bainbridge P (2013) Wound healing and the role of fibroblasts. J Wound Care 22(8):407.

- Benedetti L, Cortivo R, Berti T, Berti A, Pea F, Mazzo M, Moras M, Abatangelo G (1993) Biocompatibility and biodegradation of different hyaluronan derivatives (Hyaff) implanted in rats. Biomaterials 14(15):1154–1163.

- Biagini G, Muzzarelli R, Giardino R, Castaldini C (1992) Biological materials for wound healing. In: Brine BJ, Sandford PA, Zikakis JP (eds)

Advances in chitan and chitosan. Elsevier Science Publishers, London, pp 16–24.

- Blakytny R, Jude E (2006) The molecular biology of chronic wounds and delayed healing in diabetes. Diabet Med 23(6):594–608.

- Braun K, Prowse D (2006) Distinct epidermal stem cell compartments are maintained by independent niche microenvironments. Stem Cell Rev 2(3):221–231.

- Caplan AI, Correa D (2011) The MSC: an injury drugstore. Cell Stem Cell 9(1):11–15.

- Cha J, Falanga V (2007) Stem cells in cutaneous wound healing. Clin Dermatol 25:73–78.

- Chou T, Fu E, Wu C, Yeh J (2003) Chitosan enhances platelet adhesion and aggregation. Biochem Biophys Res Commun 302(3):480.

- Chu Y, Yu D, Wang P, Xu J, Li D, Ding M (2010) Nanotechnology promotes the full-thickness diabetic wound healing effect of recombinant human epidermal growth factor in diabetic rats. Wound Repair Regen 18(5):499–505.

- Conduit, C, Free, B, Sinha, S. TIME-H in clinical practice—a pilot study. Wound Pract Res. 2013;21:186-189.

- Dai T, Tanaka M, Huang Y, Hamblin M (2012) Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects. Expert Rev Anti- Infect Ther 9(7):857–879.

– Dazzi F, Lopes L, Weng L (2012) Mesenchymal stromal cells: a key player in ‘innate tolerance’? Immunology 137(3):206–213.

– Dickinson LE, Gerecht S. Engineered Biopolymeric Scaffolds for Chronic Wound Healing. Front Physiol. 2016 Aug 5;7:341.

– Dowsett C. Using the TIME framework in wound bed preparation. Br J Community Nurs. 2008 Jun;13(6):S15-6.

– Dumville J, Keogh S, Liu Z, Stubbs N, Walker R, Fortnam M (2015) Alginate dressings for treating pressure ulcers. Cochrane Database Syst Rev 5:CD011277.

– Eming S, Krieg T, Davidson J (2007) Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol 127:514–523.

– Falanga V, Isaacs C, Paquette D, Downing G, Kouttab N, Butmarc J, Badiavas E, Hardin-Young J (2002) Wounding of bioengineered skin: cellular and molecular aspects after injury. J Invest Dermatol 119(3):653–660.

– Falanga V. The chronic wound: impaired healing and solutions in the context of wound bed preparation. Blood Cells Mol Dis. 2004 Jan-Feb;32(1):88-94.

– Galassi G, Brun P, Radice M, Cortivo R, Zanon G, Genovese P, Abatangelo G (2000) In vitro recon- structed dermis implanted in human wounds: degradation studies of the HA-based supporting scaffold. Biomaterials 21(21):2183–2191.

– Gould LJ (2015) Topical collagen-based biomaterials for chronic wounds: rationale and clinical application. Adv Wound Care 5(1):19–31.

– Guarro G, Cozzani F, Rossini M, Bonati E, Del Rio P. The modified TIME-H scoring system, a versatile tool in wound management practice: a preliminary report. Acta Biomed. 2021 Sep 2;92(4):e2021226. doi: 10.23750/abm.v92i4.10666. PMID: 34487096; PMCID: PMC8477093.

– Gurtner GC, Werner S, Barrandon Y, Longaker MT (2008) Wound repair and regeneration. Nature 453(7193):314–321.

– Gurtner GC, Werner S, Barrandon Y, Longaker MT (2008) Wound repair and regeneration. Nature 453(7193):314–321.

– Hachach-Haram N, Bystrzonowski N, Kanapathy M, et al. A prospective, multicentre study on the use of epidermal grafts to optimise outpatient wound management. Int Wound J. 2017 Feb;14(1):241-249.

– Hadjipanayi E, Bauer AT, Moog P, Salgin B, Kuekrek H, Fersch B, Hopfner U, Meissner T, Schlüter A, Ninkovic M, Machens HG, Schilling AF (2013) Cell-free carrier system for localized delivery of peripheral blood cell-derived engineered factor signaling: towards development of a one-step device for autologous angiogenic therapy. J Control Release 169(1–2):91–102.

– Hakkinen L, Koivisto L, Gardner H, Saarialho- Kere U, Carroll J, Lakso M, Rauvala H, Laato M, Heino J, Larjava H (2004) Increased expres-

sion of beta6-integrin in skin leads to spontaneous development of chronic wounds. Am J Pathol 164: 229–242.

- Hu S, Kirsner RS, Falanga V, Phillips T, Eaglstein WH (2006) Evaluation of Apligraf® persistence and basement membrane restoration in donor site wounds: a pilot study. Wound Repair Regen 14(4):427–433.

- Ingber DE (2004) The mechanochemical basis of cell and tissue regulation. Mech Chem Biosyst 1(1):53–68 37. Nie B, Yue B (2016) Biological effects and clinical application of negative pressure wound therapy: a review. J Wound Care 25(11):617–626.

- Ingber DE (2005) Mechanical control of tissue growth: function follows form. Proc Natl Acad Sci U S A 102(33):11571–11572.

- Jerome D (2007) Advances in negative pressure wound therapy: the VAC instill. J Wound Ostomy Continence Nurs 34(2):191–194.

- Kanazawa T, Nakagami G, Goto T et al. Use of smartphone attached mobile thermography assessing subclinical inflammation: a pilot study. J Wound Care 2016; 25(4):177-80.

- Kasai Y, Morino T, Dobashi I, Mori E, Yamamoto K, Kojima H. Temporary Storage of the Human Nasal Tissue and Cell Sheet for Wound Repair. Front Bioeng Biotechnol. 2021 Jul 21;9:687946. doi: 10.3389/fbioe.2021.687946. PMID: 34368095; PMCID: PMC8334733.

- Khong PCB, Yeo MSW, Goh CC. Evaluating an iPad app in measuring wound dimension: a pilot study. J Wound Care. 2017 Dec 2;26(12):752-760.

– Kim PJ, Heilala M, Steinberg JS, Weinraub GM (2007) Bioengineered alternative tissues and hyper- baric oxygen in lower extremity wound healing. Clin Podiatr Med Surg 24(3):529–546.

– Kojima K, Okamoto Y, Kojima K, Miyatake K, Fujise H, Shigemasa Y, Minami S (2004) Effects of chitin and chitosan on collagen synthesis in wound healing. J Vet Med Sci 66(12):1595–1598.

– Lauer G, Sollberg S, Cole M, Kreig T, Eming S (2000) Expression and proteolysis of vascular endothelial growth factor is increased in chronic wounds. J Invest Dermatol 115(1):12–18.

– Leaper DJ, Schultz G, Carville K, et al. Extending the TIME concept: what have we learned in the past 10 years?. Int Wound J. 2012 Dec;9 Suppl 2:1-19.

– Lerman O, Galiano R, Armour M, Levine J, Gurtner G (2003) Cellular dysfunction in the diabetic fibro- blast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia. Am J Pathol 162(1):303–312.

– Li X, Liu J, Liu Y, Hu X, Dong M, Wang H, Hu D (2015) Negative pressure wound therapy accelerates rats diabetic wound by promoting agenesis. Int J Clin Exp Med 8(3):3506–3513.

– Ligresti C, Bo F. Wound bed preparation of difficult wounds: an evolution of the principles of TIME. Int Wound J. 2007 Mar;4(1):21-9.

– Lim K, Free B, Sinha S. Modified TIME-H: a simplified scoring system for chronic wound management. J Wound Care. 2015 Sep;24(9):415-9.

– Lindholm C, Searle R. Wound management for the 21st century: combining effectiveness and efficiency. Int Wound J. 2016;13 Suppl 2:5-15.

– Little C, McDonald J, Jenkins MG, et al. An overview of techniques used to measure wound area and volume. J Wound Care. 2009 Jun;18(6):250-3.

– Mayet N, Choonara YE, Kumar P, et al. A comprehensive review of advanced biopolymeric wound healing systems. J Pharm Sci. 2014 Aug;103(8):2211-30.

– Mayol L, De Stefano D, Campani V, De Falco F, Ferrari E, Cencetti C, Matricardi P, Maiuri L, Carnuccio R, Gallo A, Maiuri M, De Rosa G (2014) Design and characterization of a chitosan physical gel promoting wound healing in mice. J Mater Sci 25(6):1483–1493.

– McCarty S, Percival S (2013) Proteases and delayed wound healing. Adv Wound Care 2(8):438–447.

– Medical Advisory Secretariat. Management of chronic pressure ulcers: an evidence-based analysis. Ont Health Technol Assess Ser. 2009;9(3):1-203. Epub 2009 Jul 1.

– Mirzaalian Dastjerdi H, Töpfer D, Rupitsch SJ, Maier A. Measuring Surface Area of Skin Lesions with 2D and 3D Algorithms. Int J Biomed Imaging. 2019 Jan 15;2019:4035148.

– Moffatt CJ, Doherty DC, Smithdale R, et al. Clinical predictors of leg ulcer healing. British Journal of Dermatology. 2010; 162(1):51–8.

– Mostow EN, Haraway GD, Dalsing M, Hodde JP, King D, Group OVUS (2005) Effectiveness of an extracellular matrix graft (OASIS Wound Matrix) in the treatment of chronic leg ulcers: a randomized clinical trial. J Vasc Surg 41(5):837–843.

– Mustoe TA, O'Shaughnessy K, Kloeters O (2006) Chronic wound pathogenesis and current treatment strategies: a unifying hypothesis. Plast Reconstr Surg 117:35s–41s.

– Nair DG, Miller KG, Lourenssen SR, Blennerhassett MG (2014) Inflammatory cytokines promote growth of intestinal smooth muscle cells by induced expression of PDGF-Rbeta. J Cell Mol Med 18(3):444–454.

– O'Meara S, Martyn-St James M, Adderley U (2015) Alginate dressings for venous leg ulcers. Cochrane Database Syst Rev 8:CD010182.

– Okamotoa Y, Yanoa R, Miyatakea K, Tomohirob I, Shigemasac Y, Minamia S (2003) Effects of chi- tin and chitosan on blood coagulation. Carbohydr Polym 53(3):337–342.

– Pastar I, Stojadinovic O, Tomic-Canic M (2008) Role of keratinocytes in healing of chronic wounds. Surg Technol Int 17:105–112.

– Peluso G, Petillo O, Ranieri M, Santin M, Ambrosio L, Calabró D, Avallone B, Balsamo G (1994) Chitosan-mediated stimulation of macrophage function. Biomaterials 15(15):1215–1220.

– Pirone L, Bolton L, Monte K, Shannon R (1992) Effect of calcium alginate dressings on partial-thickness wounds in swine. J Investig Surg 5:149–153 73.

– Rees RS, Robson MC, Smiell JM, Perry BH (1999) Becaplermin gel in the treatment of pressure ulcers: a phase II randomized, double-blind, placebo- controlled study. Wound Repair Regen 7(3):141–147.

– Rennert RC, Rodrigues M, Wong VW, Duscher D, Hu M, Maan Z, Sorkin M, Gurtner GC, Longaker MT (2013) Biological therapies for the treatment of cutaneous wounds: phase III and launched therapies. Expert Opin Biol Ther 13(11):1523–1541.

– Reyzelman A, Crews RT, Moore JC, Moore L, Mukker JS, Offutt S et al (2009) Clinical effective- ness of an acellular dermal regenerative tissue matrix compared to standard wound management in healing diabetic foot ulcers: a prospective, randomised, multicentre study. Int Wound J 6(3):196–208.

– Romanelli M, Dini V, Bertone MS (2010) Randomized comparison of OASIS wound matrix versus moist wound dressing in the treatment of difficult-to-heal wounds of mixed arterial/venous etiology. Adv Skin Wound Care 23(1):34–38.

– Rowan MP, Niece KL, Rizzo JA, Akers KS (2017) Wound penetration of cefazolin, ciprofloxacin, piperacillin, tazobactam, and vancomycin during negative pressure wound therapy. Adv Wound Care 6(2):55–62.

– Sandoval M, Albornoz C, Muñoz S, Fica M, García-Huidobro I, Mertens R, Hasson A (2011) Addition of chitosan may improve the treatment efficacy of triple bandage and compression in the treatment of venous leg ulcers. J Drugs Dermatol 10(1):75–79.

– Santoro M, Gaudino G (2005) Cellular and molecular facets of keratinocyte reepithelization during wound healing. Exp Cell Res 304(1):274–286.

– Schultz GS, Barillo DJ, Mozingo DW, Chin GA; Wound Bed Advisory Board Members. Wound bed preparation and a brief history of TIME. Int Wound J. 2004 Apr;1(1):19-32.

– Schultz GS, Wysocki A (2009) Interactions between extracellular matrix and growth factors in wound healing. Wound Repair Regen 17(2):153–162.

– Scimeca CL, Bharara M, Fisher TK, Kimbriel H, Mills JL, Armstrong DG (2010) An update on pharmacological interventions for diabetic foot ulcers. Foot Ankle Spec 3(5):285–302.

– Sen CK, Gordillo GM, Roy S, Kirsner RS, Lambert L, Hunt TK, Gottrup F, Gurtner GC, Longaker MT (2009) Human skin wounds: a major and snowballing threat to public health and the economy. Wound Repair Regen 17(6):763–771.

– Senet P, Vicaut E, Beneton N, Debure C, Lok C, Chosidow O (2011) Topical treatment of hypertensive leg ulcers with platelet-derived growth factor- BB: a randomized controlled trial. Arch Dermatol 147(8):926–930.

– Shultz G, Davidson J, Kirsner RS, Bornstein P, Herman I (2012) Dynamic reciprocity in the wound microenvironment. Wound Repair Regen 19(2):134–148.

– Shultz G, Sibbald R, Falanga V, Ayello E, Dowsett C, Harding K, Romanelli M, Stacey M, Teot L, Vanscheidt W (2003) Wound bed preparation: a systematic approach to wound management. Wound Repair Regen 11(Suppl 1):S1–S28.

– Singer AJ, Clark RAF (1999) Cutaneous wound healing. N Engl J Med 341(10):738–746.

– Smiell JM, Wieman TJ, Steed DL, Perry BH, Sampson AR, Schwab BH (1999) Efficacy and safety of becaplermin (recombinant human platelet-derived growth factor-BB) in patients with nonhealing, lower extremity diabetic ulcers: a combined analysis of four randomized studies. Wound Repair Regen 7(5):335–346.

– Stern R (2004) Hyaluronan catabolism: a new meta- bolic pathway. Eur J Cell Biol 83(7):317–325.

– Stockton KA, McMillan CM, Storey KJ et al. 3D photography is as accurate as digital planimetry tracing in determining burn wound area. Burns 2015; 41(1):80–84.

– Stojadinovic O, Pastar I, Nusbaum AG, Vukelic S, Krzyzanowska A, Tomic-Canic M (2014) Deregulation of epidermal stem cell niche contrib- utes to pathogenesis of non-healing venous ulcers. Wound Repair Regen 22(2):220–227.

– Sun H, Pulakat L, Anderson DW. Challenges and New Therapeutic Approaches in the Management of Chronic Wounds. Curr Drug Targets. 2020;21(12):1264-1275.

– Sweeney I, Miraftab M, Collyer G (2012) A critical review of modern and emerging absorbent dressings used to treat exuding wounds. Int Wound J 9(6):601–612.

– Teot L, Ohura N. Challenges and Management in Wound Care. Plast Reconstr Surg. 2021 Jan 1;147(1S-1):9S-15S.

– Tracy LE, Minasian RA, Caterson EJ (2016) Extracellular matrix and dermal fibroblast function in the healing wound. Adv Wound Care 5(3): 119–136.

– Turner N, Kielty C, Walker M, Canfield A (2004) A novel hyaluronan-based biomaterial (Hyaff-11) as a scaffold for endothelial cells in tissue engineered vascular grafts. Biomaterials 25(28):5955–5964.

– Turner NJ, Badylak SF. The Use of Biologic Scaffolds in the Treatment of Chronic Nonhealing Wounds. Adv Wound Care (New Rochelle). 2015 Aug 1;4(8):490-500.

– Ueno H, Mori T, Fujinaga T (2001) Topical formulations and wound healing applications of chitosan. Adv Drug Deliv Rev 52(2):105–115.

– Vaalamo M, Weckroth M, Puolakkainen P, Kere J, Saarinen P, Lauharanta J, Saarialho-Kere U (1996) Patterns of matrix metalloproteinase and TIMP-1 expression in chronic and normally healing human cutaneous wounds. Br J Dermatol 135(1):52–59.

– Valente PM, Deva A, Ngo Q, Vickery K (2016) The increased killing of biofilms in vitro by combining topical silver dressings with topical negative pressure in chronic wounds. Int Wound J 13(1):130–136.

– Valle M, Maruthur N, Wilson L, Malas M, Qazi U, Haberl E, Bass E, Zenilman J, Lazarus G (2014) Comparative effectiveness of advanced wound dressings for patients with chronic venous leg ulcers: a systematic review. Wound Repair Regen 22(2):193–204.

– Wainwright D, Madden M, Luterman A, Hunt J, Monafo W, Heimbach D, Kagan R, Sittig K, Dimick A, Herndon D (1996) Clinical evaluation of an acellular allograft dermal matrix in full-thickness burns. J Burn Care Rehabil 17(2):124–136.

– Wang SC, Anderson JAE, Evans R, et al. Point-of-care wound visioning technology: Reproducibility and accuracy of a wound measurement app. PLoS One. 2017 Aug 17;12(8):e0183139.

– Weiser JR, Saltzman WM (2014) Controlled release for local delivery of drugs: barriers and models. J Control Release 190:664–673.

– Werdin F, Tennenhaus M, Schaller H-E, Rennekampff H-O (2009) Evidence-based management strategies for treatment of chronic wounds. Eplasty 9:e19.

– West D, Fan T-P (2002) Hyaluronan as a regulator of angiogenesis in wound repair. In: Tai-Ping D, Kohn EC (eds) The new angiotherapy. Humana Press, New York.

– Wong VW, Rustad KC, Glotzbach JP, Sorkin M, Inayathullah M, Major MR, Longaker MT, Rajadas J, Gurtner GC (2011) Pullulan hydrogels

improve mesenchymal stem cell delivery into high-oxidative-stress wounds. Macromol Biosci 11(11):1458–1466.

- Zhang Y, Chan HF, Leong KW (2013) Advanced materials and processing for drug delivery: the past and the future. Adv Drug Deliv Rev 65(1):104–120.

- Zhang YG, Wang X, Yang Z, Zhang H, Liu M, Qiu Y,Guo X (2013) The therapeutic effect of negative pressure in treating femoral head necrosis in rabbits. PLoS One 8(1):e55745.

- Zimmerlin L, Rubin JP, Pfeifer ME, Moore LR, Donnenberg VS, Donnenberg AD (2013) Human adipose stromal vascular cell delivery in a fibrin spray. Cytotherapy 15(1):102–108.