RESEARCH ARTICLE

Cutaneous wound healing: cellular mechanisms and therapies (an update)

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To my students who fully understand the meaning of freedom!

AP1 = Activator Protein 1
bFGF = Basic Fibroblast Growth Factor
DC = Dendritic Cells
E2F= E2 Transcription Factor
ECM= Extracellular Matrix
EGF = Epidermal Growth Factor
ERG = ETS related gene
IFN = Interferon
IL= Interleukin
MC= Mast cells
MMPs= Matrix metalloproteases
MSC = Mesenchymal stem cells
pDCs = Plasmacytoid Dendritic Cells
PGDF = Platelet Derived Growth Factor
PPARs= Peroxisome Proliferator-Activated Receptors
SCF=Secreting Stem cell factor
SMA=Smooth Muscle Actin
SMAD = Small Mother Against Decapentaplegic
STAT3 = Signal transducer and Activator of Transcription 3
TGF=Transforming Growth Factor
TGFbeta=Tranforming Growth Factor beta
TLR=Toll-Like Receptor
TNF= Tumour Necrosis Factor
VEGF=Vascular Endothelial Growth Factor

Abstract

Wound healing requires a complex interaction and coordination of different cells and molecules. Any alteration in these highly coordinated events can lead to either delayed or excessive healing. This review summarizes the principal facts that occur during wound healing including the responses of the various cell types involved and eventual therapies actually used. Interesting is also the last paragraph in which an hypothesis is considered to justify a synchronized response of the various cell types during the healing of wounds where the mast cell is considered one of the protagonists. Therefore the purpose of this review is to stimulate the reader's attention to this phenomenon and to serve as a point of reference for the various professional figures who are involved daily in dealing with the problems posed by wounds and their recovery.
Key words: Cellular Infiltrate, Mast cells, Wound Healing.

1, Structure of skin

Skin is the largest organ of the body, serving primarily as a protective barrier against the environment. It also helps to prevent body dehydration and constitutes a physical barrier, limiting the penetration of potentially harmful agents to internal organs. The skin has a three-layer structure composed of epidermis, dermis and hypodermis. The epidermis, the superficial layer, is mainly composed of keratinocytes but also contains other cell types, such as Langerhans cells and melanocytes, providing a barrier against infection and moisture loss[1, 2]. The dermal layer, situated below the epidermis, is responsible for the elasticity and mechanical integrity of the skin. It contains vascularized ECM rich in collagen, elastin and glycosaminoglycans. The cellular components of the dermal layer include endothelial cells, fibroblasts, mast cells, smooth muscle cells. The hypodermis, located below the dermis, is mainly composed of adipose tissue and collagen, and mainly acts as an energy source (For more details see [1, 2]).

2, Reasons to study wound healing

Skin wounds and compromised wound healing are major concerns for the public health sector. Complex and lengthy treatments cause an increasing burden on healthcare expenses. Even in uncomplicated cases, burns and chronic and other difficult to treat wounds require surgery and extended hospitalization periods. In the United States alone, millions of patients need treatment for chronic wounds and an estimated US billion is spent annually. More worryingly, the burden is growing year by year mainly due to the increasing prevalence of obesity and diabetes. There are more than 1,000 wound healing centers in the United States today, and wound healing has become a specialty, with fellowship programs offered at some academic centers [3]. Things do not go better in Europe: in the United Kingdom, about 200,000 patients have a chronic wound [4] and in the European Community the prevalence is expected over 1.5 million people [4, 5]. It is estimated that in Italy approximately 2 million patients a year are treated for problems relating to skin lesions, a part of whom may develop into chronic wounds with an overall prevalence around 200.000 people. The introduction of new technologies in wound care is particularly required for the treatment of complicated ulcers, dehisced surgical wounds, burns, diabetic foot ulcers and in the management of infected wounds or wounds colonized by biofilms (For more details see [3, 6]).

3, Vertebrates and wound healing

Wound healing is a conserved evolutionary process among species and encompasses spatially and temporally overlapping processes including inflammation, blood clotting, cellular proliferation and extracellular matrix secretion and remodeling. However, the outcome of wound healing in the skin differs between species. Some lower vertebrates including fish (zebrafish) and amphibians (Axolotl and Xenopus) possess the ability to perfectly regenerate skin. In contrast, it is challenging for adult mammals, including humans, to achieve such regeneration. Typically, healing of deep wounds in adult mammals results in scar tissue that lacks skin appendages. Although scar formation can meet the requirements of the skin's fundamental function in preventing infection and dehydration this process can also be unfavorable. Because of its obviously distinct appearance from the original intact skin, the scar formed as a result of injuries or burns can result in devastating cosmetic and psychological consequences, reducing the quality of life of the individual; hypertrophic and retracting scars can cause limitations to joint movements and strictures of orifices. Furthermore, skin appendages are an integral part of the skin's biological and physiological function. For example, skin epithelial appendages contribute epidermal cells for response to injury [7-11]. Additionally, the hair follicle and sebaceous gland confer additional roles for the skin as sensory and thermoregulatory organs. Consequently, scar formation prevents the complete recovery of

skin function. Thus, the ability to restore the skin to its original state is highly valued. Interestingly, studies have reported remarkable examples of scarless healing in fetal skin and appendage regeneration in adult skin following the infliction of large wounds. The models used in these studies have offered a new platform for investigations of the cellular and molecular mechanisms underlying wound healing and skin regeneration in mammals. These may provide important insights into the regeneration of missing structures and redevelopment of fully functional skin (For more details see [7-11]).

4, Early response of a body to injury

The immediate response of the body to injury is to prevent exsanguination and promote hemostasis. Damaged arterial vessels rapidly constrict through the contraction of smooth muscle in the circular layer of the vessel wall, mediated by increasing cytoplasmic calcium levels. Vessels up to a diameter of 5 micron can be completely closed through contraction, although this can only occur if the injury is in a transverse plane. Within a few minutes, the reduced blood flow mediated by arteriole constriction leads to tissue hypoxia and acidosis. This promotes the production of nitric oxide, adenosine and other vasoactive metabolites to cause a reflex vasodilatation and relaxation of the arterial vessels. Simultaneously, histamine release from MC also acts to increase vasodilatation and increase vascular permeability, facilitating the entry of inflammatory cells into the extracellular space around the wound. This explains the characteristic warm, red, swollen appearance of early wounds. Further blood loss at this stage is also prevented through the formation of a clot which is achieved through three key mechanisms:

1. Intrinsic pathway of the clotting cascade (contact activation pathway) and endothelial damage as a result of tissue injury exposes the sub-endothelial tissues to blood which results in the activation of factor XII (Hageman factor) which catalyzes the activation of FXI, which in turn activates factor IX to activate FIXa, leading to thrombin generation and fibrin formation and the formation of a fibrin plug.

2. Extrinsic pathway of the clotting cascade (tissue factor pathway) and endothelial damage results in exposure of tissue factor (which is present in most cells) to circulating blood. This results in activation of factor VII and the rest of the extrinsic pathway of the clotting cascade which eventually results in thrombin activation.
3. Platelet activation (For more details see : [12-14]).

5, Macroscopical phases of wound healing

Grossly, wound-healing analysis is characterized by the following parameters:
At wound infliction, 1 to 3 days postwounding: This stage includes blood-clot formation (primary clot), activation of epidermal edges, and early inflammatory response (characterized by abundance of neutrophils at the wound gap).

4 to 7 days postwounding: Morphologically, this stage is marked by scab formation. Histological analysis reveals migration of the epidermal edges, selective proliferation of the early granulation tissue, and inflammatory response (lymphocytes and macrophages present in abundance).

8 to 12 days postwounding: Morphologically, scab detachment is observed. Histological results exhibit the formation of new epidermis that becomes differentiated by day 12. In addition, dermal closure is initiated, concomitant to granulation-tissue formation. This stage is accompanied by attenuation of the inflammatory response.

12 to 30 days postwounding advanced healing stages: characterized by matrix remodelling, terminal differentiation of the newly formed epidermis, increased elastic-fiber content and increased wound strength (For more details see : [15-17]).

6, Acute inflammatory reaction

One characteristic of living organisms is the ability to respond in the presence of an external stimulus. When this stimulus is a traumatic offense, whether biologic, physical, or chemical, the tissue response consists mainly of an inflammatory reaction that is proportional to the magnitude of the tissue offense. The inflammatory reaction consists of a complex mechanism, the objective of which is destruction of the noxious stimulus and tissue repair.

This phenomenon was described by Hunter in 1793: "Inflammation, of any cause, is an effect destined to restore the natural function of affected parts, and it should not be considered as an illness, but a beneficial process which appears after the noxious stimulus goes down". In fact, the inflammatory reaction is a complex phenomenon that is not well understood. It consists of several apparently different processes such as enzymatic and biochemical induction, hemodynamic and vascular changes, and cellular activity, among others. All of these processes are aimed at destruction of the noxious stimulus and effecting tissue repair. The vital reaction also includes other related events which are not strictly considered part of the inflammatory response, such as platelet aggregation, complement activation by coagulation factors, and metabolism of prostaglandin. The vital reaction, as described by Strassman in 1954 and according to a previous definition by Plenk in 1786, is "... one of organs and tissues, which needs for development the presence of living cells". This kind of reaction does not happen in nonliving tissue (For more details see [18-21]).

7, Summary of principal events in wound healing

Wound healing involves a complex interaction between epidermal and dermal cells, the extracellular matrix, blood vessels and plasma derived proteins, coordinated by an array of cytokines and growth factors.

This dynamic process is classically, but somewhat artificially, divided into four overlapping phases: haemostasis, inflammation, proliferation, and remodeling.

Thrombus formation — which requires interaction between endothelial cells, platelets, and coagulation factors — achieves hemostasis after tissue injury. Trapped cells within the clot, predominantly platelets, trigger an inflammatory response by the release of vasodilators and chemoattractants and activation of the complement cascade. Inflammatory cells such as mast cells, neutrophils and macrophages create the conditions that stimulate fibroblast migration, proliferation activation, and collagen production. Together with ongoing neovascularization in the wound bed granulation tissue is formed in this proliferation phase. The combination of granulation tissue contraction by activated fibroblasts and re-epithelialization by keratinocytes then restores tissue integrity and reduces the wound size to a permanent scar. In the final maturation phase, the overall numbers of fibroblastic cells and vessels are reduced by programmed cell death. Eventually, remodeling will take place due to myofibroblasts and secreted metalloproteases. It is evident that disturbance of the fine equilibrium between cells, growth factors, and ECM in any of these phases will impact on the normal course of healing. Given the complexity of the healing process and its versatility in handling a vast variety of different wound scenarios, it is actually amazing that failure does not occur more frequently. Only if all redundancies fail to compensate disturbances or if the tissue defect is too large, cell-cell and cell- ECM miscommunications will result in either chronic or excessive healing (For more details see [16, 17, 21-22]).

7, Summary of functions of principal cells involved in wound healing

7.1, Platelets

Platelets are unnucleated fragments of bone marrow megakaryocytes. They have a crucial role in wound healing process. Not only

are they essential for clot formation, they also produce multiple growth factors and cytokines which continue to regulate the healing cascade. Over 300 signaling molecules have been isolated from activated platelets, which influence and modulate the function of other platelets, leukocytes and endothelial cells. In addition to these factors, in response to the injured cell membranes caused by the wounding stimulus, arachidonic acid is broken down into a number of potent signaling molecules such as the prostaglandins, leukotrienes and thromboxanes which have roles in stimulating the inflammatory response (For more details see [23, 24]).

7.2, Neutrophils

Neutrophils, are highly motile cells which infiltrate the wound within an hour of the insult and migrate in sustained levels for the first 48hours. This is mediated through various chemical signaling mechanisms, including the complement cascade, interleukin activation and TGFbeta signaling, which leads to neutrophils passing down a chemical gradient towards the wound, a process termed chemotaxis. Neutrophils have three main mechanisms for destroying debris and bacteria. Firstly they can directly ingest and destroy foreign particles, through phagocytosis process. Secondly, neutrophils degranulate and release a variety of toxic substances (lactoferrin, proteases, neutrophil elastase and cathepsin) which will destroy bacteria as well as dead host tissue. Recent evidence has shown that neutrophils also produce chromatin and protease 'traps' which capture and kill bacteria in the extracellular space. Oxygen free radicals are produced as a by-product of neutrophil activity, which are known to have bactericidal properties but can also combine with chlorine to sterilize the wound. (For more details see [25, 26]).

7.3, Mast cells

Mast cells (MC), contribute to wound healing. At the onset of a cutaneous injury, the accumulation of MC and release of proinflammatory and immunomodulatory mediators have been well documented. The role of MC-derived mediators has been investigated through the stages of wound healing including inflammation, proliferation, and remodeling. They contribute to hemostasis and clot formation by enhancing the expression of factor XIIIa in dermal dendrocytes through release of TNFalpha, and contribute to clot stabilization. Keratinocytes, by secreting SCF, recruit MC to the site. MC in return release inflammatory mediators, including predominantly histamine, VEGF, IL6, and IL8, that contribute to increase of endothelial permeability and vasodilation, and facilitate migration of inflammatory cells, mainly monocytes and neutrophils to the site of injury. MC are capable of activating the fibroblasts and keratinocytes, the predominant cells involved in wound healing. MC stimulate fibroblast proliferation during the proliferative phase via IL4, VEGF, and bFGF to produce a new ECM. MC derived mediators contribute to neoangiogenesis, fibrinogenesis, or reepithelialization during the repair process. MC activation inhibition and targeting the MC derived mediators are potential therapeutic strategies to improve wound healing through reduced inflammatory responses and scar formation (For more details see [27-31]).

7.4, Dendritic cells

Cellular interactions between DC and MC were previously described and this can induce the differentiation of precursors into DC through both the release of short range acting soluble factors and contact-mediating plasma membrane molecules or exosomes [32]. Recently it has been demonstrated in a murine model that wound closure was significantly delayed in DC-deficient mice DC enhancement significantly accelerated early wound closure, associated with increased and accelerated cellular proliferation, granulation tissue formation, and increased TGFβ1 levels and $CD31^+$ vessels in healing wounds. Besides in other works it has been demonstrated an increase of density of dendritic cells in response to a wound also in man. Therefore DC play an important role in the acceleration of early

wound healing events, likely by secreting factors that trigger the proliferation of cells that mediate wound healing (For more details see [33-36]).

7.5, Lymphocytes

Lymphocytes appear in the wound after 72 hours and are thought to be important in regulating wound healing, through the production of an extracellular matrix scaffold and collagen remodelling. Experimental studies have shown that inhibition of T-lymphocytes results in decreased wound strength and impaired collagen deposition. The lymphocytes exert a specific response against microbes and other foreign material in the wound: B-lymphocytes via antibodies and the T-lymphocytes through production of cytokines and stimulation of cytolytic activity. Lymphocyte-induced inflammation is then resolved by apoptosis when IFNgamma and TNFalpha are produced at the wound site (For more details see [14, 37-39]).

7.6, Macrophages

Macrophages are much larger phagocytic cells that reach peak concentration in a wound at 48 and 72 hours after injury. They are attracted to the wound by the chemical messengers released from platelets and damaged cells and are able to survive in the more acidic wound environment present at this stage. Macrophages contain in their granules a large reservoir of growth factors, such as TGFbeta and EGF, which are important in regulating the inflammatory response, stimulating angiogenesis and enhancing the formation of granulation tissue. Recently about the secretion of extracellular matrix an interaction between fibroblasts and macrophages was described. The various macrophage functions are linked to the type of receptor interaction on the macrophage and the presence of cytokines. Two distinct states of polarized activation for macrophages have been defined: the classically activated (M1) macrophage phenotype and the alternatively activated (M2) macrophage phenotype. M1 macrophages have the role of effector cells in TH 1 cellular immune responses. M2 macrophages appear to be involved in immunosuppression and tissue repair (For more details see [40-43]).

7.7, Keratinocytes

Keratinocytes are the major cellular component of epidermis, and they have several critical roles in the wound healing process. They are involved in the intricate mechanisms of initiation, maintenance, and completion of wound healing. There is ample evidence that keratinocytes stimulate fibroblasts to synthesize growth factors, which in turn will stimulate keratinocyte proliferation in a double paracrine manner. Moreover, fibroblasts can acquire a myofibroblast phenotype under the control of keratinocytes. This depends on a finely tuned balance between a proinflammatory or a TGFbeta dominated environment [44].

7.8, Plasmacytoid dendritic cells

Plasmacytoid dendritic cells (pDCs) express TLR7 and TLR9 and produce large amounts of type I IFNs in response to viral nucleic acids. However, the contribution of this rare circulating cell population to host immunity remains unclear. Recent studies now show that, although they are absent from normal skin, pDCs are rapidly recruited to sites of cutaneous inflammation. Here, they serve as an early source of type I IFN and contribute to wound healing in normal mice also through the interaction with T reg cells (For more details see [45- 47]).

7.9, Fibroblasts

Fibroblasts are critical in all three phases, playing a key role in the deposition of ECM components, wound contraction and remodeling of new ECM. Different fibroblast subpopulations with distinct functions have been identified in the skin. The functional heterogeneity of fibroblasts is not only important for skin homeostasis but also for wound healing.

Lineage tracing studies have shown that the initial wave of dermal repair following wounding is mediated by lower dermal fibroblasts, which express myofibroblast markers such as alphaSMA. These cells secrete large amounts of ECM proteins such as collagen, and abnormal collagen deposition is a feature of scarring [48]. By contrast, upper dermal fibroblasts are recruited during subsequent wound re-epithelialization, during which they are required for hair follicle formation. The available evidence suggests that delivery of upper dermal fibroblasts could be beneficial in resolving scar formation and promoting scar-free wound healing, because they produce less fibrillar collagen than lower dermal fibroblasts. Conversely, lower dermal fibroblasts could have applications in breast reconstruction in mastectomy patients because of their ability to differentiate into adipocytes (For more details see [49- 51]).

7.10, Myofibroblasts

Gabbiani et al. have established that granulation tissue contraction is actively promoted by specialized fibroblasts, which have been accordingly named ‘‘myofibroblasts’’ [52]. After restoring tissue integrity in physiological wound healing, myofibroblast activities cease and excessive cell numbers are reduced by apoptosis; the signals triggering massive cell death and the timing are not entirely clear. It is however evident that persistence of myofibroblasts activity will lead to tissue deformation by contracture. In the skin, contractures manifest as hypertrophic scars and ‘‘stiff skin’’ in the fibrotic lesions of scleroderma (For more details see [53-54]).

7.11, Pericytes

Pericytes are a newly cells recognized in wound healing. In fact these cells during wound repair communicate with endothelial cells and therefore control neo-vascularization during wound healing by regulating vessel formation and stabilization. Pericytes have been observed to possess phagocytic properties and may interact with macrophages during the inflammatory phase of wound healing. Besides, these cells can act as antigen presenting cells to promote the activation of T-cells. Pericytes secrete several cytokines and some of them were found to enhance lymphocyte infiltration. There is emerging evidence-indicating pericytes may a source of myofibroblast and transition could occur through a PDGF-dependent mechanism (For more details see [55]).

8, Molecular biology of wound healing

Wound healing is a complex process in which a variety of transcription factors and related molecules participate, including TGFbeta/Smad, E2F family, STAT3, homeobox genes, hormone receptors (androgen, estrogen, and glucocorticoid), PPARs, Wnt/beta catenin signaling, AP-1, c-Myc, and Erg1 and other factors (see table 1 of [56]). These factors are not independent but mutually associated with each other.

9, Impairment of wound healing and therapeutic strategies

Many factors can impair wound healing. Local factors include the presence of foreign bodies, tissue maceration, ischemia, and infection. Systemic factors as advanced age, malnutrition, diabetes, and renal disease may be important. In addition, reduction in tissue growth factors, an imbalance between proteolytic enzymes and their inhibitors, and the presence of senescent cells seem to be particularly important in the pathogenesis of chronic wounds. A crucial factor is tissue hypoxia, which may be caused by primary vascular diseases, metabolic diseases such as diabetes, local and systemic infection, malnutrition and persistent local pressure. In skin ulcers, the persistence of the inflammatory phase leads to high protease activity and so to degradation of growth factors and of other molecular stimuli involved in tissue repair. Growth factors may become trapped by extracellular matrix molecules or be degraded by proteases. Imbalance between hydrolases – matrix metalloproteases, elastase and plasmin

protease – and their inhibitors results in abnormal degradation of the extracellular matrix [16, 40].

Various medical approaches and therapeutic interventions can affect the different processes involved in wound healing. The healing time may be shorter when there is less injured tissue, for example during minimally invasive surgery.

Topical application of growth factors and proteinase inhibitors, incision priming with PDGF or IL-1, electrical field stimulation, use of prosthetic materials, gene and stem cell therapies can decrease healing time.

Epidermal stem cells are a convenient target to use in wound therapies because they already reside within the skin and participate in the normal healing response. They have been shown to support healing by increasing proliferation and migration of fibroblasts and keratinocytes as well as enhancing angiogenesis by human vascular endothelial cells.

Mesenchymal stem cells (MSC) therapy is another emerging option to treat acute and chronic non-healing wounds. Beneficial effects are accomplished through structural repair via cellular differentiation, immunomodulatory responses, direct secretion of growth factors, advanced neovascularization and reepithelialization, as well as mobilization of resident staminal cells. Thereby, MSC play a pivotal role in all three healing phases. At the wound margins they stimulate the formation of granulation tissue by enhancing epidermal cell proliferation and growth of new blood capillaries. Further, endothelial cell recruitment is stimulated through the release of pro-angiogenic factors and growth factors such as vascular endothelial growth factor and angiopoietin-1. MSC modify tumor necrosis factor-α production and lower NK cell function in the inflammatory phase, thereby reducing interferon-γ activity. In the last healing phase, scar formation is reduced through PGE2 secretion and lowering of TGF-β1 to TGF-β3 ratio, IL-10 up-regulation as well as IL-6 and IL-8 down-regulation. These effects are accompanied by a decline in collagen production and fibrosis. Because the delivery of MSCs through direct injection or topically through gel matrices is detrimental for cell survival and usually causes significant rapid cell death, new strategies have been developed to improve MSC cell adhesion, proliferation and migration. These techniques are based on the use of MSC-seeded micro-or nanostructured scaffolds with natural biomaterials, such as collagen and cellulose derivatives. Thereby, pronounced to complete regeneration of non-healing wounds (burns, decubitus ulcers, diabetic ulcers) has been reached in preclinical and clinical studies.

Patients with wounds often are provided pharmacologic interventions for their wounds as well as for their acute or chronic illnesses. Drugs can promote wound healing or substantively hinder it; some medications cause wound or skin reactions. The health care literature includes multiple narrative reviews describing the impact of pharmacologic agents on wound healing. Medications reported to delay wound healing include anticoagulants, antimicrobials (various antibiotic classes), anti-angiogenesis agents (eg, bevacizumab, aflibercept), antineoplastic drugs, anti-rheumatoid drugs (eg, methotrexate, aspirin/nonsteroidal anti-inflammatory drugs [NSAIDs]), colchicine (anti-gout drug), Dakin's solution (sodium hypochlorite), nicotine, steroids, and vasoconstrictors. Because of their ubiquity of use, 2 categories of medication require special mention: steroids and NSAIDs. Several literature reviews support that short-term use of both categories has limited impact on wound healing. However, long-term use of steroids and NSAIDs can have marked negative impact. Steroids are notorious inhibitors of wound healing. Noted systemic effects include hyperglycemia, osteoporosis, and mood alterations. Narrative reviews describe how steroids alter gene expression once they cross the cell membrane and thereby alter almost every phase of wound healing. Steroids decrease the inflammatory response, fibroblast activity, and epithelial regeneration and, over time, thin the epidermis and inhibit

wound contraction. NSAIDs, given long-term and especially in higher doses, can impair healing. Narrative reviews describe how NSAIDs can delay bone healing, impair ligament health, and cause serious adverse skin reactions. Selected drugs and drug categories can assist wound repair. These include hemorrheologic agents (eg, pentoxifylline), hormones (estrogen), phenytoin, prostaglandins, zinc, vitamin A, and vitamin C. Multiple narrative literature reviews support that selected "natural" medications used topically also can augment wound healing. Many have been used for centuries in a variety of cultures to assist wound healing. They include aloe vera, curcumin, ginger, medicinal (eg, Manuka) honey, mucilage (slippery elm), and witch hazel. More recently, prescription pharmacologic agents have been used off label as topical therapy to help wounds heal. They include topical calcium channel blockers, regular insulin, nitroglycerine, opioid-related drugs, phenytoin, retinoids, sildenafil, and sucralfate

Photomedicine includes both the study and treatment of diseases caused by exposure to light and on the other hand the diagnostic and therapeutic applications of light for detecting and curing disease. Light energy is capable of causing heating, mechanical effects and chemical reactions. The transfer of light energy through photon absorption can lead to many different consequences in photomedicine. Moreover, there are many new approaches for using light to see inside the body to detect and diagnose disease. Modern scientific disciplines such as biomedical optics, photochemistry, photobiology, cell biology, laser physics, and engineering have all made major contributions to the development of photomedicine as a fully-fledged division of medical science. Light can be used to detect and diagnose medical conditions even deep within the body. The therapeutic uses of light are manifold. UV phototherapy and PUVA treat many skin diseases, especially those with immune components, and lasers are used in dermatology, ophthalmology, dentistry and general surgery (among other medical specialties). The combination of harmless light with non-toxic photosensitizing dyes is used in photodynamic therapy to kill many undesirable cells, including malignant cancer cells and infectious microorganisms.

LLLT (Low Level Light Therapy (LLLT)) is an emerging medical and veterinary technique in which exposure to low-level laser light or light emitting diodes might stimulate or inhibit cellular function, possibly leading to beneficial clinical effects. The use of low levels of visible or NIR light for reducing pain, inflammation and edema, promoting healing of wounds, deeper tissues and nerves, and preventing tissue damage has been known for almost forty years since the invention of lasers. Despite many reports of positive findings from experiments conducted in vitro, in animal models, and in randomized controlled clinical trials, LLLT remains controversial. It has proposed that mitochondria are a likely site for the initial effects of light, specifically that the enzyme cytochrome c oxidase (unit four in the mitochondrial respiratory chain) absorbs photons and increases its activity leading to increased ATP production, modulation of reactive oxygen species and induction of transcription factors.

Light-emitting diodes (LED) are revolutionizing the whole lighting industry. Their availability in almost any wavelength and with steadily increasing total output power means that light delivery applications, previously thought to require an expensive laser, can now be performed at a tiny fraction of the cost (less than 1%) by LEDS compared with the equivalent laser source. Not surprisingly, LEDs are becoming much more widely used in medical applications. LEDS have several differences from lasers however. Firstly the output wavelengths are much less monochromatic than lasers, with a typical LED having a Full-Width Half-Maximum of 30 nm compared to 2 nm for a laser. Secondly LED light is non-coherent, so for LLLT applications where coherence is considered important, this may be an important difference. Thirdly, the

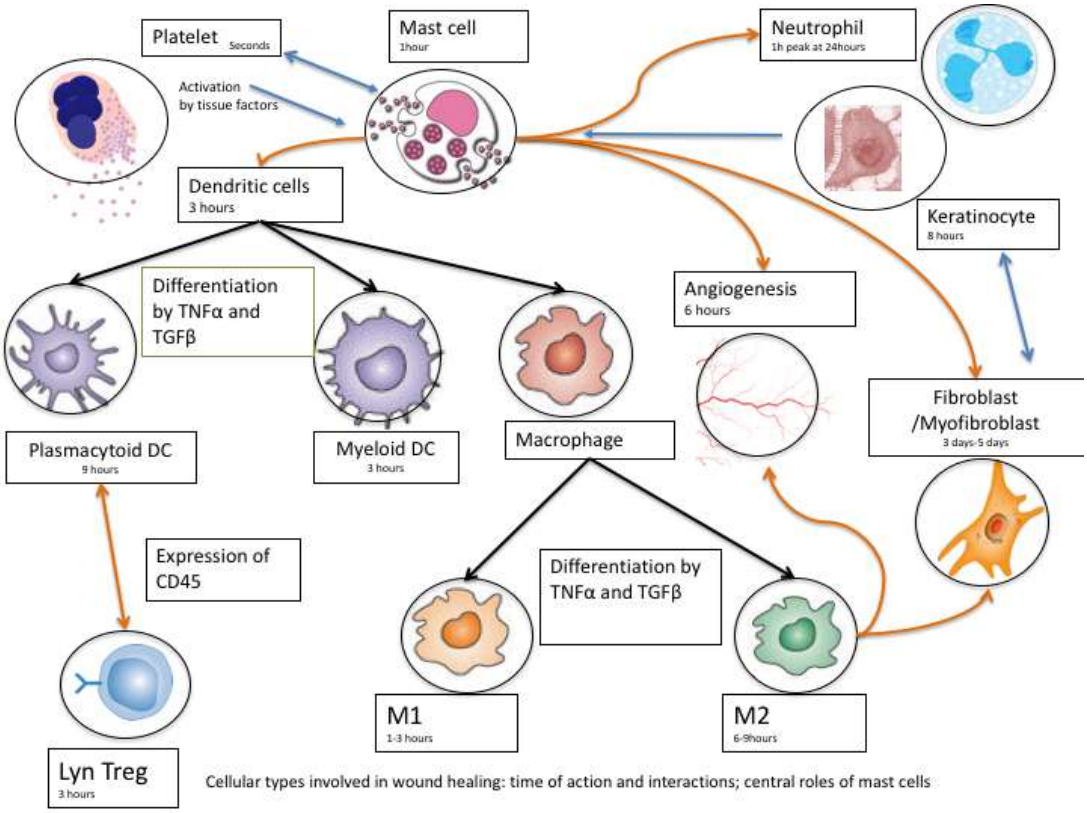
light is non-collimated, and this makes it very difficult to focus it into a fiber optic cable for endoscopic and internal applications. (For more details see : [57 – 62]).

10, Concluding remarks: central role of mast cells in wound healing

Considering the salient data of this review, mast cells are recognized as playing a central role during the processes that regulate wound healing. In summary, the activation of MC, by keratinocytes or factors in the microenvironment, leads in addition to the release of TNFalpha for the differentiation of dendritic cells, to the secretion of mediators to induce angiogenesis, to the release of extracellular matrix and to the reorganization of the cellular infiltrate.

These events could lead to secretions of TGF beta by other cells such as M1 cells. As a result of the secretion of TGFbeta already present in microenvironment, macrophages may be able to differentiate in M1 and M2 phenotypes. These cells probably in synergy with keratinocytes activate fibroblasts, which are able of differentiating within myofibroblasts [63].

The pleiotropic activity of TGFbeta appears to be correlated also to the differentiation of plasmacytoid DC that, interact together with T reg cells for a mechanism that can induce tolerance [64] via the coexpression of CD45 by these last cells [65] in wound healing processes (Fig. 1).



Cellular types involved in wound healing: time of action and interactions; central roles of mast cells

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Conflict of interests

The author declare that there is no conflict of interest regarding the publication of this article

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