Acute and Chronic Wound Management: Assessment, Therapy and Monitoring Strategies

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Anisha Kabir, Anwita Sarkar, and Ananya Barui

6.1 Introduction

Wound healing is a dynamic, interrelated and well-organized process dependent on highly regulated factors that work in synchrony to restore the structural and functional integrity of the injured tissue. The sequence of healing process works in a normal manner in the vast majority of wounds, however under underlying pathological conditions, like diabetes, it undergoes modification and leads to a delayed healing. Chronic wounds arising from the failed healing process possess a serious load on both the patient and the healthcare system. It has been estimated that nearly 6.5 million patients annually suffer from chronic wounds due to the increasing prevalence of diabetes. Treatment of chronic wounds nearly costs over US$25 billion per year to the medical system as a whole (Brem et al. 2007). Along with chronic wounds, acute and simple wounds also require proper care and attention. The process of wound healing is highly fascinating and gaining widespread interest both scientifically and commercially (Han and Ceilley 2017).

Treatment plans for both acute and chronic wounds involve invasive, non-invasive, external and internal techniques. Appropriate diagnosis, assessment and monitoring are the primary and the predominant approach to a wound care management plan. Assessment and monitoring of the healing process involve the evaluation of both the visual appearance and the detailed analysis of the internal microstructure of the wounds. Both invasive and non-invasive methods have evolved to evaluate the wound assessment rate. Several non-invasive optical imaging modalities have been currently adopted to evaluate the components of the healing wounds. Three conditions that are important to be considered during the

A. Kabir · A. Sarkar · A. Barui (✉)
Centre for Healthcare Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur, Howrah, West Bengal, India
e-mail: ananyabarui.chest@faculty.iiests.ac.in

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use of an external aid for the wound healing purpose are the patient's safety, its effectiveness and convenience of use to treat the wound (MacNeil 2007). Non-invasive and minimally invasive treatment techniques are evolving at a tremendous pace due to their targeted therapy and non-contact nature. This chapter aims to provide an insight in the recent updates and advancements in the treatments, assessment, monitoring and management of chronic and acute wounds along with the underlying physiological, cellular and molecular aspects of the wounds.

6.2 Physiology of Wound Healing

The undertaking of the wound management plans and treatments requires a prior perception of the underlying physiological, cellular, biological and molecular aspects of the complex wound healing mechanism. The overall biological phenomenon of repairing a wound site is considered as an intricate physiological network of various processes that involve two of the most vital attributes of living organisms: repair and regeneration, which ultimately results in the restoration of the integrity of the tissue. Regeneration involves the replacement of injured damaged tissue with normal cells, whereas repair involves the re-establishment of the normal functioning and integrity of the tissue. The wound healing phenomenon can be physiologically classified into four different dynamic phases which entail haemostasis or coagulation, inflammatory phase, growth and proliferative phase and remodelling (Ellis et al. 2018) (Fig. 6.1).

Haemostasis: The first and the initial step of wound healing that occurs immediately post-trauma to the skin is the constriction of the injured ends of the blood vessels to control and minimize the blood loss. Any type of injury to the skin that penetrates deep into the dermis results in the traumatization of the blood vessels, which ultimately results in haemorrhage. Haemostasis and the formation of a wound matrix are the characteristics of the initial phase of wound healing.

A series of complex chain reactions occur upon immediate exposure of the blood to air, which results in the formation of a blood clot and is termed the coagulation cascade reaction. The blood clot formed is characterized by the formation of fibrin, fibronectin and vitronectin-rich provisional matrix that temporarily fills and closes the space created due to the wound and gradually desiccates to form a scab. This provides strength and protects the wound from further infection. As the process of healing proceeds, the scab formed gets lysed along with plasmin, bacteria and several inflammatory dead cells.

The peripheral vasoconstriction of the blood vessels results in a transient hypoxic condition of the surrounding tissues. This results in increased glycolysis, alteration in the pH levels and several other factors, which stimulate and activates the coagulation cascade reaction by activating and aggregating the platelets (Ruggeri 2006). These activated platelets promote the process of inflammation via the release of mitogens and chemoattractants, which modifies and increases the vascular permeability and tone. This facilitates cell migration and increased the diffusion of oxygen and nutrients for the newly formed cells.

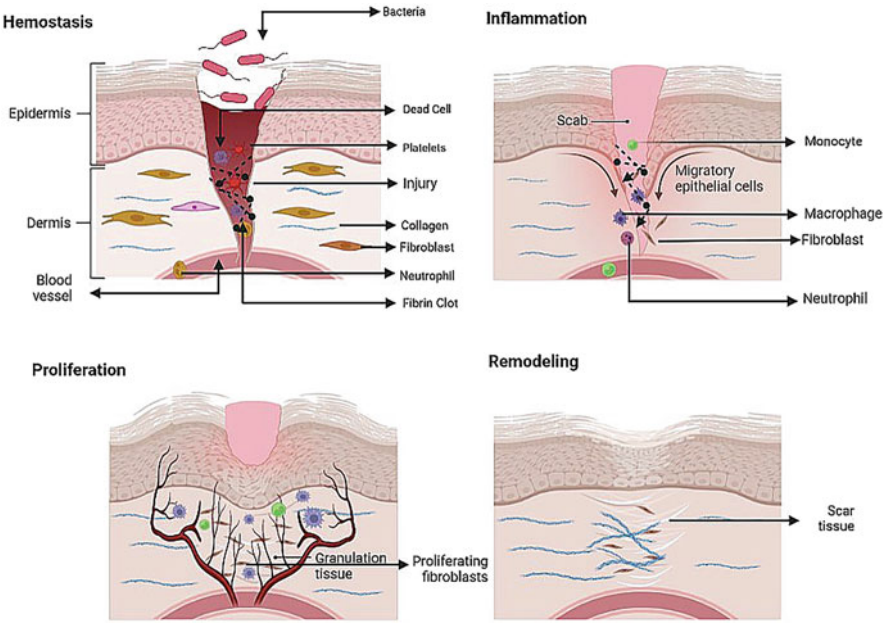

Fig. 6.1 The four phases of wound healing: haemostasis, inflammation, proliferation, remodelling; Created with BioRender.com

Inflammatory Phase: The inflammatory phase clinically termed the debridement phase is activated during the coagulation phase of wound healing. This phase of wound repair is designed to protect and prevent wounds from invading microorganisms. Secretions of inflammatory cells, growth factors, cytokines, macrophages and neutrophils function as the core of the early wound repair process. Several vasoactive mediators and chemoattractants produced by the coagulation process, injured stromal cells, mast cells and activated complement pathway recruit leukocytes at the site of trauma to initiate the process of rolling, adhesion and finally the migration of the inflammatory cells to the site of injury. Further, chemoattractants also stimulate the production of enzymes by activating neutrophils, accelerating their penetration deep into the dermis. Neutrophils are recruited and act as the first line of defence by removing debris and microorganisms through phagocytosis, followed by the production of enzymes and ROS. The process of neutrophil migration ceases after the destruction and clearance of the contaminants. The viable neutrophils die within 3–4 days and are engulfed and removed by the tissue macrophages (Singer and Clark 1999).

Macrophages perform an important role in healing wounds, by exerting inflammatory process, cytokine production and phagocytosis during the early phase of wound healing and later stimulating the proliferative phase to complete the formation of extracellular matrix (ECM). During the early phase, macrophages referred to as M1 macrophages express TNF-α (tumour necrosis factor-α) and interleukins,

which play a significant role in the recognition and killing of the pathogens. Later during the proliferative phase, it retrieves its fibrinolytic, anti-inflammatory phenotype, and is termed as the M2 macrophages. These macrophages actively signal to the dermal fibroblast and induce the process of re-epithelization and formation of the ECM. The process of inflammation halts through the universal pathway of apoptosis of the unneeded phagocytic cells that do not elicit any further inflammation (Mahdavian Delavary et al. 2011).

Growth and Proliferative Phase: The third phase of the wound healing clinically referred to as the wound repair phase is designed to protect the surface of the wound via the formation of a provisional matrix and a new epithelial cover to regain back the vascular integrity of the tissue. Formation of fibroblast, angiogenesis and epithelization are the predominant characteristics of the proliferative phase. After almost 2–7 days of the injury, migration of fibroblast and keratinocyte endothelial cells into the clot occur to form a granular tissue and replace the fibrin clot. The granular tissue formed is rich in fibronectin and hyaluronan and is characterized by a red, and granular appearance. The tissue is formed by the three elements: macrophages that protect the wound from invading microorganisms, promote angiogenesis and fibroblast formation, fibroblasts that induce the production of the ECM proteins, and new blood vessels to restore the vascular network and integrity of the tissue (Theoret 2016).

One of the key physiological processes involved in this phase is angiogenesis, that is, formation of new blood vessels and capillaries from the pre-existing ones to maintain the circulation of gases and nutrients through the newly formed cells and tissue structure. Angiogenesis occurs in response to the hypoxic condition of the surrounding tissues and is moderated by various angiogenic factors, like PDGF (platelet-derived growth factors), VGF (vascular growth factors), fibroblast growth factors, as well as several other cytokines and chemoattractants. In addition to the aforementioned metabolic phenomena, covering the denuded epithelial tissue is crucial for the successful closure of the surface of the wound (Liekens et al. 2001).

Remodelling: The final phase of wound healing also referred to as the maturation phase is characterized by the maturation of granulation tissue into scar tissue. Tensile strength of the tissue is enhanced by the random reorganization of the collagen fibres and increased cross-linking of the collagen molecule by the action of the enzyme lysyl oxidase. The initial scar tissue formed is replaced ECM, similar to the normal skin, and remodelling of ECM proteins occurs by the regulated actions of different classes of proteases.

6.2.1 Factors Affecting Wound Healing

The dynamic process of wound healing is related to multiple local and systemic factors that can lead to impairment of the process. Factors that have a direct effect on the wound properties can be called the local factors, while the overall individual health conditions fall under the systemic factors. The two most common and

important local factors that impact the process of healing involve low oxygen tension and infection of the underlying tissues (Guo and Dipietro 2010).

The most critical element that promotes wound contraction and thereby accelerates the process of healing is the level of oxygen. It promotes the process of re-epithelization, induces fibroblast formation, and thereby leads to a faster healing process. The production of various reactive oxygen species (ROS) in the polymorphonuclear leukocytes, which kills the pathogens and protects the wound from infection, is critically dependent on the oxygen level of the surrounding tissue. Disruption of vascularization, along with alteration in several factors of the surrounding wound tissues leads to temporary hypoxia. Although the fall in the oxygen level of the surrounding tissues triggers the process of healing, however, prolonged hypoxia leads to chronic and delayed wounds. Production of several growth factors and cytokines is triggered by hypoxic conditions. Cytokines like PDGF, VEGF (vascular endothelial growth factor), (transforming growth factor β) TGF-β and TNF-α (tumour necrosis factor-α) are produced and are considered as vital promoters of cell migration, re-epithelization and angiogenesis in wound healing (Rodriguez et al. 2008). Thus, in summary, hypoxia triggers the process of wound healing by releasing growth and factors and promoting angiogenesis, while a minimum oxygen level is necessary for sustaining the process of healing.

Infection of the wound is highly detrimental to the process of wound healing. Chronic and acute wounds are often colonized and contaminated with replicating and non-replicating microorganisms. Several microorganisms that are sequestered at the surface of the skin get access to the dermis upon injury of the skin. The removal of microorganisms requires the release of proinflammatory cytokines like TNF-α and interleukin-1 and often bacteria and endotoxins lead to prolonged inflammation. This chronic period of inflammation fails to heal the wound. Bacteria like *Staphylococcus*, *Pseudomonas aeruginosa* often form biofilms over the wounds, thus protecting them from the phagocytic activity of the invading neutrophils (Bjarnsholt et al. 2008). This ensures a delay in the healing process.

The systemic factors that impact and influence the healing process entails: age, stress, sex hormones, disease conditions like diabetes, jaundice, fibrosis, several hereditary disorders, nutrition, immunocompromised disease conditions like AIDS, cancer and medications. Age is considered a major factor in the delayed healing process. Several clinical studies at cellular and molecular levels have shown a delay in the healing process in aged individuals. Several factors that contribute to it are delayed T-cell infiltration, reduced phagocytic activities of the macrophages and neutrophils, delayed angiogenesis, collagen synthesis and a reduced secretion of growth factors (Swift et al. 2001). Sex hormones like oestrogen in females and androgens in males and their precursor molecules are known to have an important effect on the process of healing. Studies have shown that genes related to inflammation, regeneration, matrix production and protease inhibitors are regulated by the hormone oestrogen, and the genes for cutaneous wound healing are regulated by the hormone androgen (Hardman and Ashcroft 2008). Compromised wound healing is also related to stress. Stressors have a direct influence on the immune and endocrine systems and have been known to cause substantial delays in the process of healing.

Several medications like glucocorticoid steroids, chemotherapeutic drugs and non-steroidal anti-inflammatory drugs that are known to interfere with the process of inflammation have significant effects on the process of healing. Glucocorticoid steroids that are often used as anti-inflammatory agents are great suppressors of fibroblast and collagen synthesis. They are also known to inhibit hypoxia-inducible factor-1 (HIF-1) formation, a primary transcriptional factor required in oxygen homeostasis regulation during wound healing (Wagner et al. 2008; Hong et al. 2014). Ibuprofen, a non-steroidal anti-inflammatory drug (NSAID), widely used for the treatment of arthritis, causes reduced fibroblast production, reduced wound contraction and delayed epithelialization, thus inhibiting the healing process. Several chemotherapeutic drugs like bevacizumab, a fragment of an antibody that neutralizes VEGF, were found to be the inhibitor of the process of angiogenesis. Thus, single or multiple factors affect individual phases, contributing to impaired wound healing (Guo and Dipietro 2010).

6.3 Challenges Faced in the Wound Healing Procedure

Management of chronic wounds is a challenge to healthcare professionals, requiring the utilization of a huge number of resources in the process worldwide. In the present times, the key challenge to the treatment of wounds and the primary component of comprehensive wound care management are overcoming the factors that are the primary mediators of the impairment of wounds. Failure of wounds to heal after almost 4 weeks of proper care and management requires the reassessment of the underlying pathology of tissue and the undertaking of advanced therapeutic strategies. Chronic lower extremity ulcers like leg and foot ulcers often require advanced therapies like the use of tissue-engineered skin grafts, in combination with other processes like phototherapy and negative pressure wound therapy. These treatments alone are reported to cost about 2–3% of the healthcare budgets in the countries (Frykberg and Banks 2015). Antibiotics and antiseptics promote the process of healing in wound care management; however, the formation of antimicrobial-resistant biofilms over the wounds is a predominant challenge in wound care management.

The prolonged persistence of the proinflammatory phase and the cytokine cascade of the wound healing process stimulate the production and release of more protease. Although in acute wounds, the elevated level of protease is managed by their inhibitors; however in chronic wounds, the level of protease often exceeds their inhibitors leading to the destruction of the matrix formed. This inhibits its progress toward the proliferative phase, lengthening the inflammatory phase by attracting more inflammatory cells (McCarty and Percival 2013). Although the production of ROS destroys the microorganisms, however in the case of chronic wounds prolonged hypoxic condition and the inflammatory phase increase the production of ROS, which ultimately destroys the extracellular matrix (ECM) proteins (Schreml et al. 2010). Moreover, chronic wounds are often characterized by senescent cells

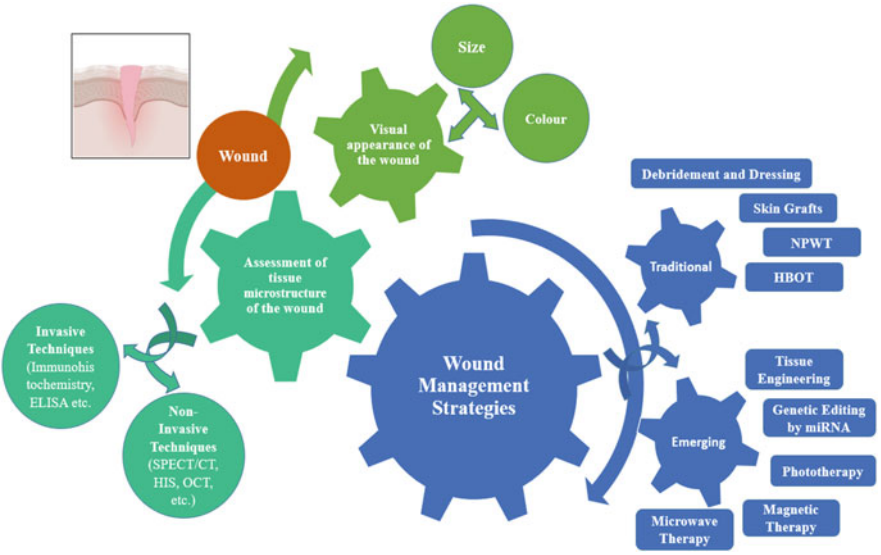

Fig. 6.2 The various steps involved in devising an effective wound management strategy

with reduced proliferative capacity and hence unresponsive to the healing signals and factors (Schultz et al. 2003).

An appropriate diagnosis for addressing the underlying etiopathogenesis of the wound is another significant challenge in the wound healing process. Proper care and management of the wound are associated with the proper and appropriate wound diagnosis and assessment (Fig. 6.2). Parallel to the management of the etiological factors of the wound, the preparation of an appropriate wound bed is also critical for the surrounding tissues. Thus, along with proper wound management, holistic or clinical treatment of the patient must also commence alongside.

6.4 Therapeutic Strategies

6.4.1 Traditional Methods

6.4.1.1 Debridement and Dressing

The most common method to treat a wounded area is to sterilize the place, remove dead tissues, or debride the area and externally apply a wound dressing to close the open wound site for its proper healing. This helps to shield the internal area from the outside environment, which enables faster healing. Traditionally used dressing materials like cotton and wool have given way to more advanced dressings, which are incorporated with therapeutic materials to aid in faster healing. The re-epithelization phase requires a moist environment, therefore dressings made of hydrocolloid, hydrogels etc. provide better results than the traditional materials

(Miller and Nanchahal 2005). Debridement of the wounds can either be achieved by dressings that help in enzymatic reactions for fibrin degradation in the wounds (Mulder et al. 1993) or surgical procedures for the removal of dead tissue to inhibit pathogenic infection and expose healthy tissues for regeneration. Natural biological enzymes from the maggot larvae have also successfully been used for the treatment of necrotic wound tissues (Thomas et al. 1999). Wound dressings are also imbibed with a number of antibiotics like tetracyclines (Anjum et al. 2016), quinolones (Ye et al. 2018) and cephalosporins (Rădulescu et al. 2016). They help reduce the bacterial load in the wounded area by either disruption of the bacterial cell wall synthesis or inhibiting protein or nuclei acid formation by disrupting the various pathways involved. With the growing number of antibiotic-resistant strains of bacteria emerging, various natural substitutes are being tried to be implemented for their antimicrobial properties (Das and Horton 2016). Essential oils (Aumeeruddy-Elalfi et al. 2016), honey (Scagnelli 2016) etc., have been successfully shown to have sufficient antibacterial properties, which may be exploited alone, or in combination with other therapeutic methods, like nanotechnology to effectively treat wounds without causing antibiotic resistance. Chronic wounds, where the healing is impeded by a lack of growth factors, may be provided externally with growth factors such as Rh-PDGF (recombinant human PDGF), TGF-β (Miller and Nanchahal 2005) to accelerate the healing process.

6.4.1.2 Skin Grafts

The use of skin grafts has been an established treatment mode for various skin injuries. Depending on their composition, they may be either epidermal, consisting of only the epidermal layer, full-thickness comprising of both the epidermal and the dermal layers, or split-thickness, made up of the epidermal and a partial dermal layer. While split-thickness grafts provide better results at large injuries with poor circulation, full-thickness grafts are more preferable for exposed body areas that require a consideration of aesthetic healing due to lesser contraction (Sun et al. 2014). Major cells used in the epithelial grafts consist of keratinocytes, which help enhance the cell proliferation in the wounds for effective healing (Kanapathy et al. 2017).

Based on their source, skin grafts may be classified into autografts, allografts and xenografts. Autografts are obtained from a different body part of the same patient whose wound is being treated (Janeway et al. 2001). While an obvious advantage is the lesser chances of rejection by the patient body, it is a painful procedure and often for large injuries unsuitable due to the limited source (Rowan et al. 2015). Allografts are obtained from either cadavers, or other living human beings, which can help in angiogenesis and vascularization, along with promoting immune cell production in the wound area of patients to assist in healing. However, the cost factor is a point of consideration while going for allogeneic grafts. Xenografts are skin grafts obtained from a different species, mainly porcine skin (Halim et al. 2010), which may be used as a temporary wound covering to help in skin regeneration in wounds. However, due to species differences, there is an issue of graft rejection, which in major parts can be solved by their genetic modification (Yamamoto et al. 2018). Another rich source of collagen and growth factors which have been effectively used in skin grafts

is amniotic membrane obtained from the placenta of various donors. Amnion membrane dressing used on burn patients has shown an adequate re-epithelization rate leading to faster wound healing with lesser pain (Eskandarlou et al. 2016).

6.4.1.3 Hyperbaric Oxygen Therapy

Hyperbaric oxygen therapy, as the name insinuates, is a therapeutic method, which includes the inhalation of 100% oxygen by the patient in an artificially created hyperbaric atmospheric chamber, that is, where the pressure is higher than the sea-level pressure (Howell et al. 2018). One of the major hindrances that compromise the healing potential in wounds leading to their chronic nature is the hypoxic environment created in it, which aids in the growth of pathogen (Mustoe 2004). Collagen formation and deposition, immune response and angiogenesis all require oxygen, and hence using the hyperbaric oxygen therapy may lead to an increase in the above, leading to wound repair (Hopf et al. 2005). A study measuring transcutaneous oxygen tension in diabetic patients with lower extremity wounds showed positive improvement of the wounds following this therapy (Fife et al. 2002). However, hyperbaric oxygen therapy might not be a suitable method for all; therefore, the wound aetiology along with patient-specific factors needs to be considered before its application. Apart from being used solely for treatment purposes of various wounds, hyperbaric therapy provides a synergistic effect when coupled with other healing treatments, such as debridement and the use of proper dressing, or skin grafts. It has been extensively used in the treatment of necrotizing fasciitis to lessen the healing time and increase the patient survival rate (Jallali et al. 2005). It has shown promising results in the treatment of foot ulcers in diabetic patients with combination to other wound care management therapies (Duzgun et al. 2008). However, a recent study on the effects of this therapy on acute injuries in rabbits did not have any significant improvement on the wound healing capacity, as otherwise stated in literature (Tlapák et al. 2021). Therefore, further evaluations need to be performed for its efficient use in wound management.

6.4.1.4 Negative Pressure Wound Therapy (NPWT)

The use of mechanical forces to modulate injuries and tissue regeneration has been a well-studied area of research for quite a while. Negative pressure wound therapy (NPWT) refers to the use of negative pressure in a controlled environment for increasing the wound healing rate. Even before its official approval to be used as a clinical therapy, the use of the core principle of NPWT dates back centuries. Its current major application is in the treatment of acute surgical wounds, along with burn patients and chronic wounds. NPWT has been shown to drastically reduce the number of times, the wound dressing needs to be changed, along with improvement of circulation in patients, thus it is the most effective therapy method for acute level burn patients as an immediate treatment (Banwell and Musgrave 2004). Due to its reports of being able to increase vascularization in the wound beds, NPWT is further used to prepare the wound beds of patients who would receive grafts (Saaiq et al. 2010). This led to a decrease in the graft rejection rate in burn patients (Scherer et al. 2002). It has shown major success in the treatment of wounds in the upper

extremities (Shine et al. 2019), which makes it a viable treatment option as a follow-up therapy for reconstruction surgeries. The traditional method requires the presence of a gauze or foam below the permeable membrane to provide suction pressure to the wounds, which along with being slightly uncomfortable has a tendency to be infected with pathogens in the process. To overcome this problem, a study group has developed a NPWT delivery method using a single layer dressing without the requirement of the gauze, which has successfully been shown to have improved wound healing potential (Nuutila et al. 2019). In comparison to treatments using standard dressing, studies on surgical injuries treated using NPWT have shown a lesser incidence of surgical site infection (Masters et al. 2021). Though limited by its high cost and requirement of learning to properly dress the wounds for this therapy, its advantages make this therapy extremely prevalent in clinical therapies for wound management, particularly in soft tissue and burn injuries. In light of the lesser number of studies with NPWT as compared to other therapies, more research studies need to be conducted to fully understand its mechanism of action at the molecular level to better optimize different treatment procedures for different types of wounds.

6.4.2 Advanced and Emerging Methods

In the recent years, more and more therapeutic methods are being developed to give way to better wound healing properties with minimal scarring. In this regard, non-invasive to minimally invasive methods are gaining more popularity due to their innate nature of targeting the wound area without much requirement of a surgical procedure.

6.4.2.1 Tissue Engineered Grafting

Skin grafts of natural origin have been used since the premodern times to treat skin injuries. However, owing to the inadequacy of donor skin graft source for more serious injuries, or its unavailability due to some pathological conditions, tissue-engineered grafts present a suitable alternative. Tissue engineering of the skin refers to the process of growing the skin cells, or the keratinocytes, at a rate faster than the normal in vivo process, and using it at the wounded area to promote healing. As an initial treatment process, though the wound can be covered with an engineered graft from synthetic materials, for the continuation of the regeneration process, angiogenesis and vascularization are essential; hence, living keratinocyte cells are required.

Various factors that are required for the proper design of a tissue-engineered scaffold are choosing suitable types of cells, materials for the scaffold, and the different types of nutrients required for their growth. Biodegradable and bioresorbable materials have proven to be more effective than synthetic materials for the construction of the scaffold, which enables angiogenesis for the proper development of the vascular system of the engineered grafts (Olson et al. 2011). Major biomaterials used for clinical engineering of grafts are agarose (Garzón et al. 2013), collagen (Meuli et al. 2019), hyaluronic acid (Galassi et al. 2000) etc. Among these, the most frequently used collagen is combined with other biomaterials such as

fibrin and hyaluronic acid. The skin surface is made up of the epidermal and the dermal layer, therefore tissue-engineered grafts may be a substitute for each individual layer, or a combination of the two. They may also be acellular, or cellular, based on the presence of cells. Examples of acellular grafts available commercially include Alloderm® (Gordley et al. 2009), Biobrane® and Integra® (Shevchenko et al. 2010). Cellular grafts consist of cells that may be autologous, or allogeneic in origin. They have been seen to provide better results as shown by a study using keratinocyte seeded hyaluronic acid membrane grafts on full-thickness wounds in nude mice wound models (Horch et al. 2019) In the recent times, newer combinations of biomaterials are being tested for better skin regeneration capabilities to increase their efficiency. Keratinocytes seeded on a fibrin-agarose scaffold were tested in vivo on nude mice models, which showed promising results (Carriel et al. 2012).

Though tissue-engineered constructs have considerably improved over the years, further studies need to be done on the development of a complete scaffold that would be functional with fully-developed blood vessels, nerves and proper pigmentation to include the aesthetic look of the graft. Further, in the recent times, 3D-bioprinted skin substitutes may provide a more effective solution (Ishack and Lipner 2020). However, the cost of implementation also needs to be optimized for covering a wider patient pool.

6.4.2.2 Genetic Editing (miRNA)

Human body consists of millions of coding and non-coding RNAs that serve a variety of functions to maintain normal physiological functions. The coding RNAs, as the name suggests, code for a variety of proteins responsible for different biological functions. The non-coding RNAs can further be divided into long and short non-coding RNAs, based on the number of nucleotides. They have been found to have important roles in posttranscriptional regulation of different proteins and signalling pathways. miRNAs (miRs) are a type of short non-coding RNAs of about 22–25 nucleotides long, which have a major role in the regulation of gene expression by degrading mRNAs, and thus preventing the protein formation. Initially transcribed by RNA pol 2 in the nucleus, the miR is processed and exported to the cytoplasm where it is further processed by RNase enzymes. The final product forms the RNA-induced silencing complex (RISC), which then helps bind to the 3′ untranslated region of complementary target mRNA strands for disruption in translation and their ultimate degradation. miR-based gene silencing has led way to an increasing interest for its potential use in the tissue repair mechanism for the treatment of chronic wounds. Chronic wounds are a result of either direct characteristics of the wound site, or an indirect result of the overall health of an individual. Few of the major markers of chronic wounds are loss of re-epithelization, reduced mitogenesis and migration potential in keratinocytes and skin fibroblast cells. In this regard, miRs have been found to be either upregulated or downregulated, which points to their critical role in various stages of tissue repair response mechanism during the different phases of wound healing.

The inflammatory phase requires a fine balance of pro- and anti-inflammatory signalling mechanisms, which may be upset due to an irregularity in miR biogenesis.

An in-depth study of over 200 miRs revealed that several of them, like miR-132, 146a/b and 155 were found to be regulated by the presence of proinflammatory cytokines and endotoxins in the cell, a typical response during inflammation (Taganov et al. 2006). This helps them in regulating the expression patterns of various genes like interleukin-1 receptor-associated kinase 1 (IRAK), Src homology 2 domain-containing inositol 5-phosphatase (SHIP1) and cyclooxygenase-2 (COX2), which are implicated in the immune response of the cell. Several miRs have also been found to have anti-inflammatory roles. miR-223 has a negative regulatory effect on the transcription factor Mef2c for myeloid progenitor formation and granulocyte differentiation, thus regulating the inflammatory response (Johnnidis et al. 2008). miR-203 is present in abundance in the keratinocytes of inflamed skin and represses the formation of proinflammatory cytokines TNFα and IL24 to inhibit immune responses required for wound healing (Primo et al. 2012).

One of the key events required for proper proliferation and angiogenesis phase is the ample formation of keratinocyte and their migration. Post-transcriptional control studies of miR-198 host gene follistatin-like 1 (FSTL1) showed a downregulation of miR-198 levels by TGFβ in keratinocytes of wounded areas, which helped form FSTL1 protein (Sundaram et al. 2013). miR-198 upregulation leads to impaired keratinocyte proliferation and prevents wound closure (Wang et al. 2015). Other miRs implicated in the keratinocyte migration for wound healing by regulation of various signalling pathways include the miR-99 family members, which act by regulating AKT/mTOR signalling pathway to aid in wound healing (Jin et al. 2013), and miR-4516 which reduces the keratinocyte motility by fibronectin/integrin alpha9 signalling pathway regulation (Chowdhari et al. 2017). Studies of wounded diabetic mouse model have revealed a myriad of miRs that aid in the angiogenesis process by targeting various signalling pathways involved in wound closure and re-epithelization, like miR-26a (Jiang et al. 2020), miR-135a-3p (Icli et al. 2019b), miR-615-5p (Icli et al. 2019a) and miR-4674 (Icli et al. 2020).

Collagen deposition during the remodelling phase of wound healing has been found to be aided by miR-29 expression in systemic sclerosis patients (Maurer et al. 2010). miR-192 and miR-29b/c also have been found to help in the scar-free healing process by enhancing collagen expression by targeting TGF-β, SMADs and SMAD-interacting protein 1 (SIP1) (Kato et al. 2007). A list of the different miRNAs is provided in Table 6.1 which play important roles in different phases of wound healing.

In view of the role of miRs in the wound healing process, it is evident that changes in their expression levels are key to an impaired wound healing process. A comparison of the miR expression profile of normal and chronic wound healing revealed major changes in the upregulation and downregulation patterns on many miRs (Banerjee et al. 2011), which point to their possible use as therapeutic agents to aid in the healing process. Though proper delivery vehicles need to be incorporated for this nucleic acid therapeutic treatment, these molecules can be detected as biomarkers in the serum, which make the process of safe optimization and dose determination easier than other molecules for similar therapeutic purposes, thus making it a potential treatment to promote healing of chronic wounds. However,

Table 6.1 A list of miRNAs used alone, or in combination with other methods in wound healing

miR	Function in wound healing	Reference
miR-132	Decreased chemokine production; suppressed NF-κB pathway. Helps in proliferation	Li et al. (2015)
miR-146 a/b	Endotoxin-responsive; targets NF-κB pathway	Taganov et al. (2006)
miR-155	Inhibition reduces inflammation in wounds; promotes healing	Ye et al. (2017)
miR-223	Negative regulatory effect on immune response; its downregulation enhances cell proliferation and nerve regeneration.	Johnnidis et al. (2008); Zhang et al. (2021)
miR-203	Repress formation of proinflammatory cytokines; inhibit immune responses	Primo et al. (2012)
miR-198	Upregulation leads to impaired keratinocyte proliferation	Sundaram et al. (2013)
miR-99	Regulating AKT/mTOR signalling pathway—keratinocyte migration	Jin et al. (2013)
miR-4516	Reduces keratinocyte motility by fibronectin/integrin alpha9 signalling pathway regulation	Chowdhari et al. (2017)
miR-26a	Inhibits healing by reduced keratinocyte migration	Jiang et al. (2020)
miR-135a-3p	Targets p38 signal pathway to reduce angiogenesis	Icli et al. (2019b)
miR-615-5p	Angiogenesis and granulation	Icli et al. (2019a)
miR-4674	Wound closure and angiogenesis	Icli et al. (2020)
miR-29	Inhibits TGF-β1/Smad/CTGF pathway; lesser scar formation	Guo et al. (2017)
miR-192	Enhances collagen expression; promotes scar-free healing	Kato et al. (2007)

accumulation in the liver and kidney, leading to their toxicity and ultimate damage, is a potential risk factor involved in this process. Therefore, a careful consideration of other health implications needs to be taken into account to be able to put this treatment at a larger scale.

6.4.2.3 Phototreatment

Since the demonstration of the biostimulatory role of low-level laser light on cells as early as in the 1970s by Mester et al. (Mester et al. 1971), various light-based therapeutic methods have been explored for their potential use for chronic wound treatment. The traditional treatment method using antibiotics for wound dressing has led to an increase in multidrug-resistant bacterial infection in the wound site, leading to their chronic nature. The non-invasive and effective germicidal activity of ultraviolet light on the wound site bioburden, in addition to their lesser adverse effect on the host tissues, has led way to their potential use in wound treatment.

Phototherapy is the utilization of polarized light for wound treatment by the proliferation of keratinocytes via triggering the cellular and humoral defence mechanisms (Fenyö 1984). Studies on the effects of treatments using low-level laser (660 nm) in the early phase rat pleurisy model as a coherent light source revealed their anti-inflammatory effect by a dose-dependent modulation of immune response molecules like interleukins and TNF-alpha. (Boschi et al. 2008) Thus, it is progressively being used in the treatment of chronic wounds and burn patients to minimize pain, promote tissue regeneration and wound healing. Additionally, studies revealed their role in increasing human gingival fibroblast proliferative capacity with a small exposure time (Almeida-Lopes et al. 2001). Treatments of slow-healing wounds using polarized polychromatic non-coherent light source have also shown significant results by increasing macrophages and neutrophils, thereby increasing bacterial phagocytosis in the wound site (Monstrey et al. 2002). The basic principle of photodynamic therapy is that photon molecules combine with several photosensitive non-toxic dyes specific to microbial cells in the wounded area to effectively increase reactive oxygen species (ROS) production, and promote their death. A comparative study to understand the more efficient method of wound treatment using polarized light as opposed to a low-level laser light source in burn patients revealed that the former had better results owing to their biostimulatory capacity to produce a cascade reaction that helps in a better healing process (Mowafy et al. 2021).

Studies of the effect of varying intensities of light emitting diodes (LEDs) showed an increase in cell growth of mouse fibroblasts and human epithelial cells in vitro, while reducing the wound size, pain and healing time in vivo (Whelan et al. 2004). Following this study, it can be hypothesized that a combined exposure to varied wavelengths would result in an enhancement of the healing process. Studies with diabetic ulcer rats treated with 660 nm and 890 nm combined LED radiation proved the same by boosting the healing process by increased ulcer granulation (Minatel et al. 2009). A recent study using a combination treatment of 630 and 940 nm LED therapy in pressure injury patients also exhibited an improvement in arterial and venous circulation resulting in an accelerated healing (Baracho et al. 2021). Further, cutaneous open wound treatment using phototherapy combined with photodynamic therapy may be a viable treatment option and be a major improvement over the utilization of the individual treatment procedures (Sampaio et al. 2021).

Near-infrared (NIR) activated nanomaterials composed of either inorganic molecules or polymers have also gained popularity for their use in wound treatment by producing local hyperthermia in the wound site for killing pathogens. Yang et al. have designed a NIR-triggerable hollow $Cu_{2-X}S$ nano-homojunction (nano-HJ) platform with covalently attached hyaluronan for their specific binding to wound sites to promote cutaneous wound healing (Gao et al. 2021). This has opened up vast possibilities for the use of nano-homojunction platforms as delivery vehicles for treatment in difficult-to-access areas in our body. Copper sulphide nanodots with antimicrobial peptides have recently been designed and successfully used in another study as a NIR-activable antimicrobial platform for wound treatment (Wang et al. 2022). Addition of collagen to this mix indicated further improvement in the healing.

Recently, a wireless LED patch design of adequate flexibility with an Internet of Thing (IoT) healthcare platform has been proposed for wound healing (Phan et al. 2021). Though the wavelength and exposure time need to be further optimized, it can be considered to be an important contribution in the biomedical field for improving the ease of access and use, along with the improved quality of cost-effective and non-invasive wound healing treatment procedures. Thus, the non-invasive nature of phototherapy provides a viable option for its use in the treatment of various wounds.

However, with the large number of positive results gained using phototherapy, more in-depth studies need to focus on the risks involved in their clinical use. Blue light irradiation studies in a scratch wound healing model were found to reduce keratinocyte migration and proliferation processes, which are important for the re-epithelization phase (Denzinger et al. 2021). Thus, a critical evaluation of the wavelength, dosage and exposure time needs to be optimized for phototherapy studies for their efficient clinical use.

6.4.2.4 Magnetic Therapy

An interesting technique in alternative medicine is the use of magnetic field for the improvement of the circulation and hence the promotion of wound healing. Electromagnetic therapies have been effectively used in bone healing since the 1950s for the treatment of fractures (Sharrard 1990), osteotomies (Fredericks et al. 2000) etc. Studies of their use in soft tissue healing started more than a decade back which showcased the improvement in healing of cutaneous wounds by their exposure to a low-power, static magnetic field (Henry et al. 2008). Cell migration, one of the key players in wound healing, is aided by the cells moving toward the injury site following the cellular cytoskeletal fibres, or the concentration gradient by sensing the changes in the internal electromagnetic fields. Understanding their effects and their potential manipulation may thus be a potential therapeutic measure in regenerative medicine. Diabetic patients, treated with the static magnetic field, have shown a significant improvement in anti-inflammatory gene expression via the JAK/STAT pathway, angiogenesis and re-epithelization, which led to faster wound closure (Shang et al. 2019).

Magnetic therapy can also be used to externally manipulate iron-oxide-based extracellular nanovesicles as delivery agents used in regenerative medicine. Human mesenchymal stem cell (MSC)-derived exosomes loaded nanoparticles in a clinical skin injury model have shown promising results for effective external navigation using the magnet to the in vivo injury site to promote angiogenesis and cell proliferation (Li et al. 2020b). Dynamic magnetic field may also be used in this regard. Extremely low-frequency electromagnetic force (ELF-EMF) exposure of chronic ulcers showed anti-inflammatory and angiogenic effects, with increased collagen formation, hinting at their multitude role in the various wound healing phases (Costin et al. 2012). They further increase ROS production (Calcabrini et al. 2017), cytokine release and upregulate matrix metalloproteinase-9 (MMP-9) expression (Ayuk et al. 2016) to help in cell migration and phagocytosis of pathogens in the wound area via the Atk/ERK pathway (Patruno et al. 2018).

Since the establishment of its role in wound healing, magnetic therapy is seen as a promising addition to traditional treatment procedures to aid in wound healing. However, more in-depth studies need to be conducted on the various factors like exposure type (local or overall), exposure duration and wound type that affect the modulation of the healing process using the magnetic field. Further, for the effective inclusion of this therapy as a mainstream treatment procedure, a more effective method of delivery with portable equipment needs to be developed for its more accessible use.

6.4.2.5 Microwave Therapy

A drawback of the use of light in a therapeutic wound management method is its inability to penetrate deep into the tissues. Various forms of electromagnetic radiations at a low frequency have been used in the medical field for a long time due to their therapeutic effects on the biological tissues. The neuromuscular system utilizes electrical signals to perform different biological functions, and hence electromagnetic radiation may be utilized to control them. Of them, the use of microwaves has emerged as a beneficial option for the treatment of various diseases due to their higher thermal ability, along with better penetration into tissues. Microwaves have a frequency range of 300 GHz to 300 MHz, with wavelengths ranging from a meter to a millimetre, on the electromagnetic spectral scale. Due to their ability to produce physiological changes, or localized heating effect to destroy diseased tissues with a minimally invasive procedure, they have long been used for tissue ablation purposes in the field of oncology. Their frequencies can be modulated to suit the required purpose, for example, a lower frequency range is used for ablation of a large amount of tissue, whereas a higher frequency range on a smaller surface area produces better results. Early studies in the mid-90s have demonstrated the physiological effect of microwaves on wound healing on the immune system by aiding in the process of WBC accumulation and phagocytosis of pathogens in the wound site, leading to faster wound closure than the control groups (Korpan et al. 1994). Further, at specific frequencies, they cause an increase in blood flow and tissue metabolism at the injury site to promote their healing (Wyper and McNiven 1976). A recent study with burn model rats revealed that short nanosecond-pulsed microwave application of low frequency at the burn site promotes faster healing (Samoylova 2020). With proper optimization, this may be applied to the human burn patients to promote wound healing with less scarring and a minimally invasive procedure. Hydrogel dressing prepared using microwave-inducible materials provides an interesting solution for targeted transdermal wound treatment with minimal invasion. A novel levofloxacin-loaded hydrogel dressing prepared using polymerization of microwave-triggerable material has recently been successfully designed with optimal delivery to the wound site, along with bactericidal properties (Gao et al. 2022). This has opened up newer avenues for developing novel biomaterial drug delivery systems which may externally be triggered for treatment of hard-to-reach wound sites.

Microwave therapy is emerging as a viable option for wound treatment due to its non-invasive nature, along with its ability for precise targeting in the deep tissues.

With miniaturization of treatment devices for ease of access, further studies need to be conducted for optimal design of portable devices to help with treatment of various types of wounds.

6.4.2.6 Nanotechnology for Wound Therapy

Nanoparticles provide an excellent method of drug delivery owing to their small size, coupled with their ability to be surface modified to reach specific targets. Therefore, it is of no surprise that they are extensively used to optimize the drug delivery process to wound sites. Along with their targeting ability, use of various biomaterials for their construction leads to further added advantages. A common example is the use of silver or zinc nanoparticles for their antibacterial properties and acceleration of wound healing. In this regard, various studies have been conducted by using different forms of nanoparticles to provide a better alternative to already available methods. Utilizing their targeting abilities, studies conducted using nanoparticle-coated stem cell provided selective targeting of the nanocarrier-coated cell toward the wound site (Liu et al. 2016). Carboxymethyl chitosan nanoparticles loaded with bioactive peptide were shown to mediate wound healing without any scarring, owing to a controlled release of the drug, along with overcoming the enzymatic environment inside the cell (Sun et al. 2018). Utilizing their antibacterial properties, various types of hydrogel dressings have been developed to treat burn wounds or chronic wounds. A collagen dressing with zinc oxide nanoparticles and plant essential oil was synthesized that gave a faster healing rate, coupled with adequate biocompatibility and low toxicity rate (Balaure et al. 2019). Silver nanoparticles have been a common choice as a drug delivery vehicle and have been commercially manufactured on hydrogel dressings to accelerate healing (Fong et al. 2005). Addition of nanoparticles to polymers such as gelatin or chitosan are a preferred model of wound healing dressing due to their ability to prevent sepsis in the wound area along with re-epithelization abilities (Hamdan et al. 2017). A study conducted using chitosan-based hydrogels, in which silver nanoparticles and calendula extract were added as an alternative to traditional drugs, showed satisfactory results to treat diabetic wound patient model (Rodríguez-Acosta et al. 2021). Injectable europium oxide nanorod (Eu_2O_3 NRs) reinforced nanocomposite hydrogel dressing has recently been developed, which displays impressive anti-inflammatory capacity by TNF-alpha and interleukin inhibition, and enhanced angiogenic potential (Luo et al. 2021). In another study, a novel polymer film combined with modified chitosan–curcumin nanoparticles was tested for skin tissue regeneration on burn patients, which showed a significantly better regenerative capability due to their synergistic effect than the individual treatments (Basit et al. 2021). Drug-loaded nanoparticles may also be used with a combination of other therapies, such as photodynamic therapy, to overcome the poor solubility issues associated with various drugs. Pulsed photomodulation of curcumin-loaded iron-oxide nanoparticles was seen to provide better wound closure abilities, along with lowering the bacterial load on the wound site, than the control groups, or only laser, or only drugs groups (Moradi et al. 2019). Thus, these properties may further be

studied and developed to exploit in creating appropriate wound therapies to treat chronic injury patients with poor natural healing capacities.

6.5 Assessment and Monitoring Wound Healing

The assessment of the wound begins during the initial and first encounter with the patient, which includes the understanding of the general appearance of both the wound and the patient. Visual inspection of the wound involving, depth, size, odour and general appearance provides immediate information about certain features of the wound. This further helps in the evaluation and treatment of the wound. Determination of the depth of penetration of the wound and tracking of several underlying associated disease conditions like osteomyelitis is possible with probing the wound (Grayson et al. 1995). Other than visual observation, evaluation of the detailed information of the microstructure and components of skin, undetectable to the visual inspection is crucial for the clinicians to assess the intensity of the injury and its healing ability, thereby developing therapeutic actions.

6.5.1 Invasive Assessment Techniques

The common invasive ancillary techniques that are utilized for the assessment and monitoring of the components of healing wound are special stains, immunohistochemistry, and enzyme-linked immunosorbent assay (ELISA). Masson's trichrome staining provides a standardized system to analyse and demonstrate the collagen content of the healing wound (Lee et al. 2012; Pişkin et al. 2014). Several immunohistochemical markers have been utilized to exhibit constituents of a healing wound. These include the use of CD31 for angiogenesis, antiloricrin for epithelial differentiation, and various antibodies against cytokine ligands and receptors. Surgical wound assessment techniques that include wound drains and wound scoring have been utilized for the quantitative and qualitative analysis of the healing wounds. Multiple stages of the healing process involving the components of the skin are evaluated for the quantitative analysis of the healing wounds. Histological parameters, which include epidermal closure, differentiation, migration, granulation tissue formation, inflammation dermal closure, re-epithelization and collagen deposition, are evaluated to provide insights into the defects of healing stages (Gupta and Kumar 2015).

6.5.2 Non-invasive Assessment Techniques

Imaging the structural alterations of the underlying wound tissues to monitor the anatomic, physiologic and mechanical progress of the healing of the wound is crucial for the management of the process. The common imaging modalities that are used to monitor various wound parameters involve CT (computed tomography),

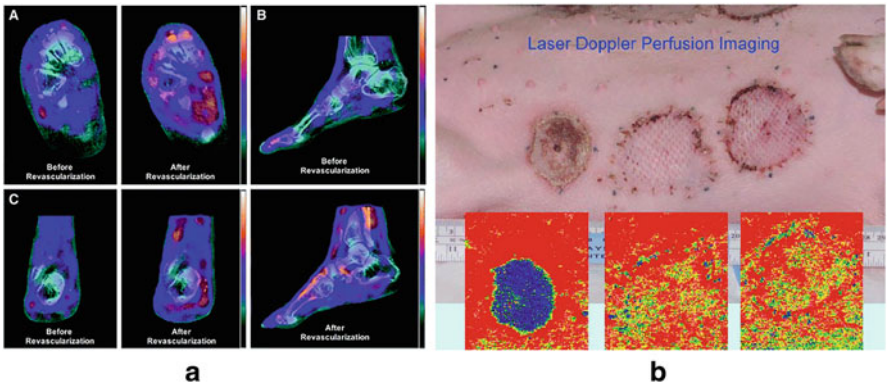

Fig. 6.3 (**a**) The SPECT/CT perfusion imaging in a critical limb ischaemia patient before and after revascularization. (A) Axial, (B) sagittal, and (C) coronal views of the foot by fused SPECT/CT demonstrate increased radiotracer uptake and better microvascular perfusion. [Reproduced from (Chou et al. 2020)]. (**b**) Laser Doppler perfusion imaging of full-thickness sulfur mustard injuries in a weanling pig model after 8 days of surgery. [Reproduced/adapted from (Graham et al. 2005)]

MRI (magnetic resonance imaging), THz (TetraHertz) spectroscopy, ultrasound imaging, SPECT/CT (single photon emission/computed tomography) (Mukherjee et al. 2017). The primary objective of the imaging databases is to improve the evaluation of wound pathogenesis and develop an effective wound treatment plan (Fig. 6.3). The joint and bone pathology of the surrounding wounded region can be assessed by a CT scan. CT angiography is utilized to obtain three-dimensional visualization of the peripheral vasculature of diabetic foot ulcers (Elsayed, Elsayed et al. 2018). SPECT/CT uses a SPECT gamma scanner along with traditional CT to produce highly sensitive, high-resolution images of the regional microvasculature of the wounded tissues to evaluate the peripheral artery diseases in limb ischaemia (Chou et al. 2020). Due to the enhanced sensitivity and resolution of MRI over CT, it is preferred both as an imaging modality and a good surgical aid in osteomyelitis of chronic wounds (Cohen et al. 2019). THz spectroscopy is utilized to detect the tissue hydration gradient of normal and injured tissue due to its unique penetrability.

Various optical non-invasive imaging techniques that have shown potential in imaging of the wound include HSI (hyperspectral imaging), OCT (optical coherence tomography), thermal imaging, laser Doppler imaging (LDI), spatial frequency domain imaging, NIR imaging spectroscopy and fluorescence imaging. These tools enable the gathering of the broad spectrum of wound parameters, which includes structural and chemical components of the tissue, oxygen and moisture level, skin blood flow, collagen deposition and re-establishment and infection (Li et al. 2020a). Digital camera imaging is a widely used non-invasive imaging method for recording the size and depth of the wound. HSI is another optical imaging modality utilized to quantify the oxygen, moisture and haemoglobin content of the wound (Lu and Fei 2014). On the other hand, thermal imaging is utilized to evaluate the depth of burn in case of burn wounds. Thermal imaging or thermography is also used to quantify the thermal diffusivity of the wounded tissue and thereby reveals the extent and stage of the healing process. The skin blood flow of the wound

Table 6.2 Different types of imaging techniques currently used for non-invasive wound assessment

Imaging mode	Uses in wound assessment	Image produced using
CT	Wounds in bones and various joints; 3D vasculature imaging of wound area	X-rays
SPECT/CT	Regional microvasculature of the wounded tissues	Gamma rays
OCT	Wound dimension, epidermal migration, vascular structures and effects, epithelization; high resolution but lesser penetration rate	Near IR rays
MRI	Highly sensitive imaging; used as surgical aid; highly penetrative	Strong magnetic field and radio waves
THz	Tissue hydration gradient detection	Terahertz waves
Ultrasound	Blood flow speed; high resolution; cost-effective; fast, real-time imaging	Ultrasound waves
Digital camera imaging	Wound size and depth measurement; poor specificity	Digital camera with 3.0 megapixels or higher resolution
HSI	Oxygen, moisture, haemoglobin content quantification of the wound; poor penetration rate	Light source (halogen lamps or light-emitting diodes)
Thermography	Thermal diffusivity of the wounded tissue; gives an idea about the phase of the wound; burn depth; poor specificity and accuracy	IR radiation measurement using IR camera
LDI	Skin blood flow measurement; less accurate	Laser beam
NIR imaging	Oxygen, haemoglobin, moisture content quantification; burn wound depth measurement; high resolution but poor specificity	Near IR rays
Spatial frequency domain	Saturation of oxygen measured; vessel structures; autofluorescence detection; time-consuming	Incoherent monochromatic light

is quantified using the unique imaging modality known as laser Doppler imaging. LDI evaluates the variation in the wavelengths of the reflected and scattered radiation after confronting the tissue and the moving RBCs (red blood cells). NIR imaging spectroscopy also quantifies the oxygen, haemoglobin, moisture content of the wound by measuring the maximum light absorbing capacities of the different components of the wound tissues. Although digital camera imaging, NIR spectroscopy and thermal imaging provide an easier and simple imaging modality, however due to their poor specificity, HSI and OCT are currently widely used imaging technique to image the internal microstructure of the tissue. A brief summary of the various types of available imaging techniques for wound assessment has been given in Table 6.2.

6.5.2.1 OCT Imaging

Optical coherence tomography (OCT) is a non-invasive diagnostic tool that has been widely and currently used in the clinical diagnosis and monitoring of the structural changes of the wounds, which include dimensions, migration of the epidermis, vasodilation and vasoconstriction, and epithelization during the process of healing (Li et al. 2020a). OCT utilizes the principle of low coherence interferometry to obtain high-resolution images of the internal network and microstructure of the tissue. OCT angiography has great potential in the quantification of the area and contraction rate of the wound (Deegan et al. 2018). PS-OCT referred to as a polarization-sensitive OCT is widely used in the determination of the functional insights of the tissues, specifically wherein the density and orientation of the collagen are significantly altered. PS-OCT can also be used to determine the birefringence of the tissue and the retardation of the phase of collagen deposition in the ECM (extracellular matrix). Multi-functional PS-OCT is used to evaluate and compute the phase retardation and the relative orientation of the axis of the tissue collagen fibres during the healing process (Park et al. 2018). Currently, the PS-OCT is utilized to monitor the optic axis, for evaluating the collagen content correlated with a particular volume of collagen orientation in the fibrotic and scar tissue (Fig. 6.4). Fourier domain-optical coherence tomography (FD-OCT), namely spectral-domain OCT (SD-OCT) and swept source OCT (SS-OCT) are utilized for the histological evaluation and clinical assessment of the wound healing process. FD-OCT images the surface and sub-surface of the wound and reveals the internal modifications of the tissue microstructure crucial for the monitoring and management of the wound. OCT employs the use of backscattered near-infrared photons from the tissues and reconstructs the image of the internal microstructure using interferometry. However, OCT images suffer a loss of intensity with depth, which limits the application of OCT to only monitoring of the cutaneous wounds, and

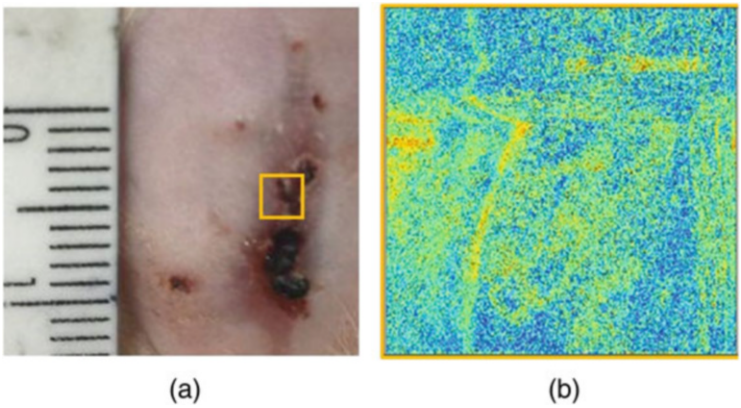

Fig. 6.4 (**a**) PS OCT imaging of scab region (indicated by square) of a wound. (**b**) En face birefringence map calculated from the PS-OCT image. [Reproduced from (Sowa et al. 2016)]

hence correction of the attenuation loss is highly crucial to effectively visualize and differentiate the stages of the healing process (Ghosh et al. 2021).

6.5.2.2 High-Frequency Ultrasound Imaging

Ultrasound imaging is widely considered a good non-invasive technique for the evaluation of chronic wounds due to its higher safety, higher spatial resolution, low cost and lower operating time compared to other imaging modalities. Ultrasound utilizes acoustic pulses to generate two-dimensional images of the tissue cross-sections. Regions with small density variations like the scar tissues appear dark in ultrasound, while regions with significant density variations appear as bright images (Li et al. 2020a). High-frequency ultrasound (HFU) skin scanners with a frequency greater than 20 MHz have been used in the clinical assessments of the dermal burns, imaging of skin microstructure, visualization of the modifications of the wound structure, evaluation of the mechanical properties, and assessment of the healing status of both acute and chronic wounds. High-resolution images with shallow depth of penetration are obtained by HFU, which further can be employed by the clinicians to develop therapeutic actions. HFU imaging is mainly employed for the evaluation and assessment of pressure ulcers, monitoring the wound status and healing progress of the underlying tissues as it does not interfere with the healing process. 2D-B mode HFU non-invasively images the surface and sub-surface of the wounds and reveals the depth and volume of the scar tissue, blood clot, content of collagen, irregularity and inhomogeneity of the tissue, and granulation tissue formation (Mohafez et al. 2018).

6.5.3 Wound Healing Models and Quantitative Analysis of the Wounds

The evaluation of the process and standardization of the quantitative analysis of the healing wounds require the establishment of several healing models. Quantitative analysis involves the evaluation of various wound parameters, like the length and width of the wound, its projected surface area, perimeter and volume. The most commonly selected technique for the delineation of the wound perimeter is the tracing method using acetate or polyurethane films. Computer-assisted planimetry is the most standardized technique utilized the clinicians to delineate the surface area and the perimeter of the wound. The simplest technique for the evaluation of the wound depth involves the use of a sterile blunt-tipped rod. The mathematical formula to assess the volume (V) of the wound was developed by Kundin using wound surface area (A) as: $V = A*D*0.327$, where A = length (L)* Width (W)* 0.785 (Kundin 1985). Alginate moulds are employed to evaluate the volume of the wound by weighing or the displacement of water. Stereophotogrammetry evaluates the wound depth by viewing the wound from two different angles and also allows the measurement of the surface area, contour and perimeter of the wound. (Langemo et al. 2001). Standardized photography evaluates the various parameters of the healing wound non-invasively.

Several wound healing models both 2D and 3D have been established to understand the healing process and generate healing products required to heal different types of wounds. 2D scratch assay, in which a wound is created by scratching a layer of confluent cells on a substrate to study the migration of the cells, is a well-established model. A mathematical model established by Lemo et al. provides a standardized model to determine the contraction of the wound and scoring of the healing process (Lemo et al. 2010). This model is based on five parameters that involve L (length of the re-epithelialization zone), S (distance between the borders of the wound), D (depth of the wound), T (thickness of the connective tissue) and N (thickness of the natural dermis on both sides of the wound). Three indices namely SCI (superficial contraction index), the DCI (deep contraction index) and the WCI (wound contraction index) are determined from the given parameters, which allow the measurement of the wound contraction. Karamichos et al. developed a three-dimensional wound healing model to study the external culture condition effects on implanted fibroblast cells (Karamichos et al. 2009). The results obtained from varying the different culture components and parameters may be utilized in future to optimize the culture conditions for increased cell growth. Another 3D model by Zhou J Chen et al. closely resembles the wound bed and its environment, allowing to observe the fibroblast migration rates on varied cell types, along with the effects of growth factors in this environment (Chen et al. 2014). Further modifications on this model by variations of different cell types and factors would lead to a better understanding of the in vivo process. Thus, these models help to understand the underlying healing process in an environment that closely imitates the wound environment in vivo.

6.6 Conclusion

Development of more potent healing strategies requires a thorough knowledge of the wound microenvironment and the molecular interactions taking place within it. Undertaking of the treatment plans and management of the wound healing phenomenon involve the prior understanding of the wound aetiology and the complex physiology of the process that constitutes haemostasis, inflammation, proliferation and remodelling. Multiple factors affect the process that include oxygen, infection of the underlying tissues and systemic factors like underlying pathological conditions like diabetes. The traditional treatment plans involve debridement and dressing of the wounds, skin graft therapy and hyperbaric oxygen therapy. Advanced non-invasive treatment techniques, tissue engineering, genetic editing and photo treatments provide targeted healing wounds with minimal scarring. The limitation of light source as a therapeutic agent in penetrating deep inside the wounded tissue is replaced by microwave and magnetic therapy. Assessment and monitoring of the healing wounds utilizing both invasive and non-invasive modalities have provided a better management approach of the healing process. Several wound healing models have been established to provide both the qualitative and quantitative analyses of the healing wounds.

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