Prasun Kumar · Vijay Kothari *Editors*

Wound Healing Research

Current Trends and Future Directions

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About the Editors

Prasun Kumar is presently working as an Assistant Professor at the Department of Chemical Engineering, Yeungnam University, Republic of Korea. He holds a Ph.D. in Biotechnology from CSIR-Institute of Genomics and Integrative Biology, Delhi, India. His main areas of research are biopolymers, microbial biodiversity, bioenergy, microbial biofilms, quorum sensing, quorum quenching, and genomics. He has over seven years of experience in applied microbiological research including over 2 years postdoctoral research experience as BK-21 plus fellow at Chungbuk National University, Republic of Korea. His research work is oriented towards valorization of lignocellulosic biowastes into value-added products and antibiofilm compounds. To his credits, there are over 29 articles in various peer-reviewed SCI journals including Trends in Microbiology, Biotechnology Advances, and Bioresource Technology. To date, his work has fetched decent citations with an h index of 24 and an i10 index of 33. He has been contributing to scientific society by actively reviewing articles for more than 32 SCI journals and was awarded the peer review award by Publons in the year 2018. He serves as the editorial board member of few international journals and also worked as the guest editor for the journals, namely 'Polymers' and 'Frontiers in Bioengineering and Biotechnology'.

Vijay Kothari is a microbiologist. His primary research interest is AMR (antimicrobial resistance). His group is actively involved in investigating antimicrobial/anti-virulence potential of natural products as well as synthetic compounds. In recent past, his lab has extensively investigated the anti-pathogenic activity of various traditional medicine formulations, e.g. *Panchvalkal*, *Panchagavya*, *Triphala*, etc., against different antibiotic-resistant bacterial strains including the wound-infective species. His lab was awarded SRISTI-DBT-BIRAC Appreciation Award for validation of anti-infective potential of a traditional polyherbal formulation—Herboheal. He has also been awarded two AIMS (Artificial Intelligence Molecular Screen) award projects by Atomwise Inc., USA for identifying novel anti-infective leads. Dr. Kothari has also contributed substantially to the field as an active editor and reviewer, and has been conferred the *Sentinel of Science* award by Publons in 2016 recognizing his contribution as a peer reviewer.

Part I
Cellular and Physiological Aspects of Wound Healing

Classification of Wounds and the Physiology of Wound Healing

Ankit Gupta

Abbreviations

Ang	Angiopoietin
BM-MSC	Bone marrow mesenchymal stem cell
CTGF	Connective tissue growth factor
DAMPs	Damage-associated molecular patterns
DETC	Dendritic epidermal T-cell
ECM	Extracellular matrix
EGF	Epidermal growth factor
FGF-2	Fibroblast growth factor-2
GPCR	G protein-coupled receptor
HGF	Hepatocyte growth factor
IGF	Insulin-like growth factor
IL	Interleukin
LC	Langerhans cells
MC	Mast cell
MMP	Matrix metalloproteinases
MMP-2	Matrix metalloproteinase-2
NETs	Neutrophil extracellular traps
PA	Plasminogen activator
PAI	Plasminogen activator inhibitor
PARs	Pattern recognition receptors
PDGF	Platelet-derived growth factor
PGE2	Prostaglandin E2
TGF-α	Transforming growth factor-α
TGF-β	Transforming growth factor-β

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TNF-α	Tumor necrosis factor-α
tPA	Tissue plasminogen activator
TRM	CD+ resident memory T-cells
TRM	Resident memory T-cells
uPA	Urokinase plasminogen activator
VEGF	Vascular endothelial growth factor.

1 Introduction

Skin is the outermost covering of the body that is frequently exposed to external stress, pathogens, etc. and acts as a barrier against the outer environment (Rinnerthaler et al. 2015; Wong et al. 2016; Gallo 2017; Losquadro 2017; Gravitz 2018). It shields internal tissues and organs of the body and provides protection against mechanical stress, microbial infection, fluid imbalance, maintains thermal dysregulation, and also permits the sensations of touch, heat, and cold (Richmond and Harris 2014; Belkaid and Tamoutounour 2016; Chen et al. 2018b; Choi and Di Nardo 2018; Kwiecien et al. 2019; Kabashima et al. 2019). As the skin is subjected to a range of external and internal pressures, it is susceptible to various types of injuries or damage. When the integrity of the multiple layers of skin, mucosal surfaces, or organ tissue is lost due to any mechanical force (such as accidental or intentional etiology), disease, or microbial infection, etc., it leads to cellular damage and the occurrence of wound (Kujath and Michelsen 2008; Wilkins and Unverdorben 2013; Putnam et al. 2015; Gonzalez et al. 2016; Obagi et al. 2019; Herman and Bordoni 2020). In other words, loss in the skin, mucosal membrane, or tissue integrity due to internal or external factors is called as wound (Kujath and Michelsen 2008; Sarabahi and Tiwari 2012; Wilkins and Unverdorben 2013; Putnam et al. 2015; Herman and Bordoni 2020). As the skin is constantly subjected to a variety of stress factors, several kinds of immune cells, including Langerhans cells (LCs), γδ T-cells, regulatory T-cells (T_{reg}), and resident memory T-cells (T_{RM}) are recruited into the skin, which plays a vital role in sustaining the physiological homeostasis (Hikosaka and Wurtz 1989; Liu et al. 2016; Ono and Kabashima 2016; Sorg et al. 2017; Kabashima et al. 2019). The basic underlying architecture of the skin and various immunological barriers present in the skin has been highlighted in Fig. 1.

The occurrence of wounds allows the entry of bacteria, viruses, or external chemicals into the body, which in turn can reason inflammation and can reason local infection (wound infection) or systemic infection (septicemia) (Percival 2002). This is a potential threat to the human organs, body, and sometimes can also lead to life-threatening conditions. Recent reports suggest that every year worldwide, scores of people are susceptible to irregular wound healing that in turn leads to long-term recovery, due to improper treatment, and moderately effective wound healing therapies (Fife and Carter 2012; Leavitt et al. 2016; Sen 2019; Rodrigues et al.

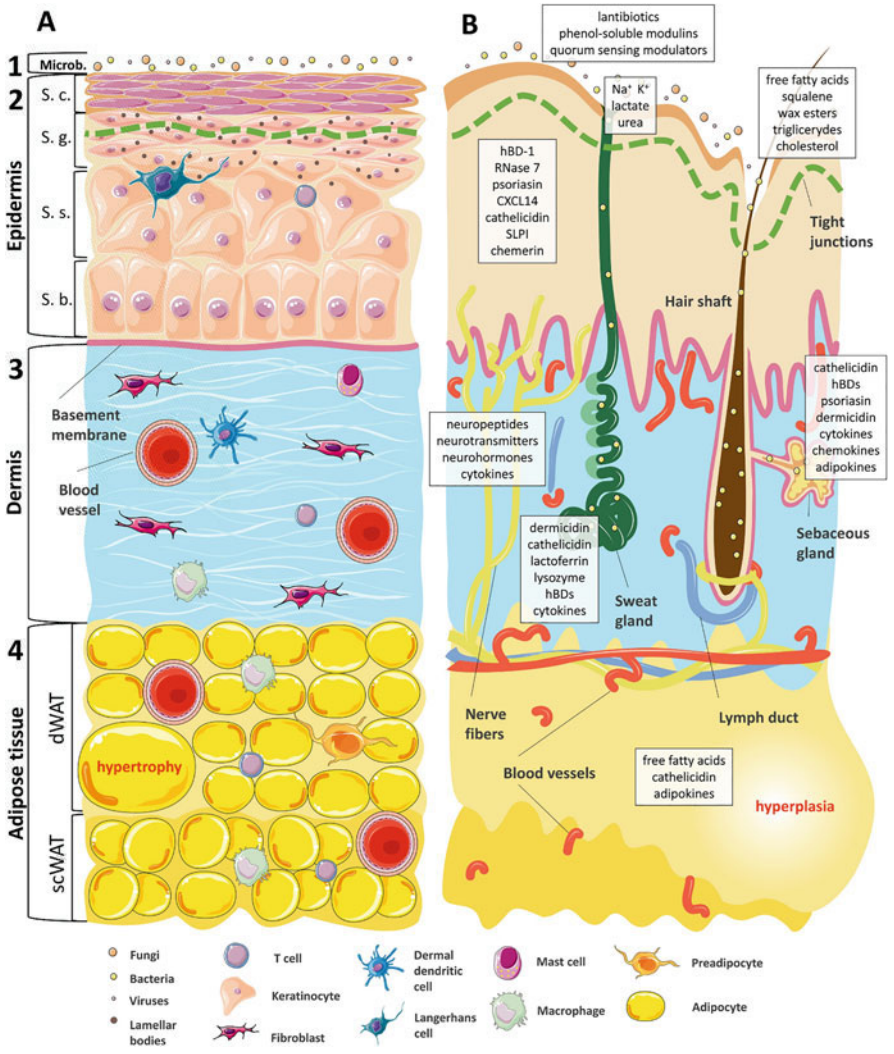

Fig. 1 The basic architecture and immunological barrier of the skin. (**a**) Structural outline of the varying layers of the skin and its components. The first layer consists of microbes such as bacteria, fungi, and viruses located at the top of the skin surface, followed by hair follicles and sweat glands. This forms the first line of defense by producing antimicrobial factors. The second layer is the epidermis which comprises four distinct strata and possesses the keratinocytes and other immune cells that are triggered upon infection. The layer below this, known as the dermis, is enriched in leukocytes and also serves as a reservoir for immune cells recruited through the bloodstream. The lowermost layer of the skin is called the adipose tissue and subdivided into white and subcutaneous white adipose tissue. This layer predominantly contains lipid-dependent immune cells and also provides defense by secreting antimicrobial peptides and factors. (**b**) Functional overview of each layer of skin corresponding to the reference mentioned in (**a**). The figure is adapted from Kwiecien, K. et al. 2019, Cytokine Growth Factor Rev. (Kwiecien et al. 2019)

2019). Therefore, wound care and rapid wound healing are critical and have clinical significance, and therefore more effective therapies of wound healing are needed.

From the early period of human life, the human body is continuously exposed to various types of injuries and diseases. Therefore, humans have always tried to find easy and effective ways of wound care and wound healing (to stop bleeding, minimize microbial infection on wounds, and in accelerating the healing process), for the successful treatment of different types of wounds (Gottrup and Leaper 2004; Broughton et al. 2006; Shah 2011; Sarabahi and Tiwari 2012; Jones 2015). Wound care has evolved over thousands of years and its treatment is an ancient area of specialization in medical science. Through evolution, the wound healing ability by regeneration of organs was replaced by repair through inflammation and subsequent deposits of the matrix proteins at the wound site (Sarabahi and Tiwari 2012). Wound care has evolved from ancient Greek medical practice (460–136 BC) through the middle ages (476 AD–1453) to the modern era of wound care (fifteenth century to twenty-first century) (Sarabahi and Tiwari 2012). During this period, we have always tried to achieve rapid wound healing to prevent wound infection and to avoid other clinical complications (Sarabahi and Tiwari 2012). The recent developments in the branches of cellular pathology, human physiology, molecular biology, microbiology, surgery, polymer chemistry, and allied fields have helped us substantially in understanding the basic mechanism of the wound healing process. With this knowledge, clinicians are now trying to find new protocols/methods of wound care and wound healing (Sarabahi and Tiwari 2012).

Wound healing is a multifaceted biological process that requires the intricate collaboration of various cell types and their products in sequential steps (Kujath and Michelsen 2008; Sorg et al. 2017; Kabashima et al. 2019; Rodrigues et al. 2019). During this process, several types of immune cells are recruited at the wound site in response to micro-environmental conditions caused by inflammatory challenges (Singh et al. 2017). These cells and chemical factors help in maintaining homeostasis upon the inflammatory challenges (Singer and Clark 1999; Gurtner et al. 2008; Eming et al. 2014; Martin and Nunan 2015; Oishi and Manabe 2018; Lim et al. 2019; Rodrigues et al. 2019). At the same time, the local environment at the site of infection changes with the improving health status of the individual. It is critical that physicians understand the fundamental physiological processes involved in the proper treatment of any cellular damage or any wound. Understanding the physiology of a typical trajectory of infection and healing through different phases guides the way for comprehending the basic principles of wound healing. With the help of this knowledge, health care professionals can develop the necessary skills to care for a wound, and the body can accomplish the complex task of tissue repair. Additionally, successful wound healing should be prioritized to reduce morbidity arising out of improper wound management (Kujath and Michelsen 2008; Gurtner et al. 2008; Singh et al. 2017).

In this chapter, we talk about two key aspects of wound healing, which are (i) classification of various types of wounds, and (ii) the cellular physiology of wound healing. Here, we first discuss the classification of these wound types in detail, which is fundamental to identifying a particular wound. Proper identification

of the wound is required for a planned and successful treatment of any patient without spreading any further contamination/infection and damage to the wound or the patient's body. Next, we discuss the cellular basis of wound healing and provide detailed information on different stages of wound healing. We also provide a detailed role of the several cell types and chemical factors involved in this process. Finally, we summarize our current understanding of the fundamental mechanism and the physiology of wound healing.

2 Classification of Wounds

Various types of trauma can damage the organ tissues and lead to the occurrence of the wound. Once the body tissues are damaged, proper wound care is required to achieve complete wound healing. In wound care, we ensure that wounds are appropriately cleaned, dressed, and treated to stop further infection (Kujath and Michelsen 2008; Wilkins and Unverdorben 2013; Onyekwelu et al. 2017; Herman and Bordoni 2020). In wound care, the first and crucial step is to identify the wound and wound type. For efficient wound management, one requires detailed knowledge of the reason of tissue damage and occurrence of a wound, likelihood of surgical site infection, characteristics of the wound, and wound type (Vu et al. 2009; Levy et al. 2013; Mioton et al. 2013; Herman and Bordoni 2020). This is possible only when we have a demarcation between various wound types or injuries, and the wounds are properly classified into different classes. Wound classification is also required because the wounds of a healthy person and a diseased person (a person having diabetes, malnutrition, patients receiving grafts, or any other disorder) shows a drastic variation in bacterial contamination, infection, as well as they may also show a differential ability to achieve wound healing (Percival 2002; Vu et al. 2009; Jones 2015; Onyekwelu et al. 2017; Herman and Bordoni 2020).

Proper classification of wounds can be achieved, when one can accurately predict the probability of postoperative complications, surgical site infections, and reoperation (Mioton et al. 2013; Herman and Bordoni 2020). Several medical practitioners have attempted wound classification, but the disparities observed across various injuries and associated wounds make it a challenging and complex process (Belkaid and Tamoutounour 2016; Choi and Di Nardo 2018; Rodrigues et al. 2019). The main problem with wound classification is the low inter-rater reliability of wounds among medical professionals (Levy et al. 2013; Onyekwelu et al. 2017; Herman and Bordoni 2020). Further, wound classification does not effectively work in neonatal surgical wounds and chronically ill children, and it requires a different classification scheme for this demography (Baharestani 2007; Vu et al. 2009; Herman and Bordoni 2020). Once the details of wound characteristics (based on their etiology, morphology, skin integrity, stage of infection, etc.) have been documented properly, we have to plan for an optimized treatment to avoid further contamination and damage to the wound or patient's body. This is required to select a suitable treatment protocol at the time of diagnosis. Thus, it is necessary to classify

wounds in several groups or subgroups for their proper identification, as described below.

2.1 Classification of the Wounds According to Etiology

Based on the homeostatic response of the wound, it becomes important to classify them. The severity of the wound depends on the mechanism of injury and any understated comorbidities of the patient. The first mode of classification discussed herein is the classification of wounds based on etiology. Etiology is nothing but the characterization of the wounds based on the comprehensive assessment of the cause of the injury (Gamelli and He 2003; Spanholtz et al. 2009; Kuhajda et al. 2014). Some wounds appear easy for the clinicians to classify, whereas some may represent more complicated tasks to identify. Assessing the root cause of the problem and rectifying it is also critical in preventing a recurrence of the wound. Thus, intentional or unintentional wounds depending on the severity, are classified into several different types, as described below (Table 1). The four main types of wounds, when classified by etiology, are trauma wounds, burn injuries, penetrating injuries, and surgical wounds.

2.2 Classification of the Wounds According to the Rank-Wakefield System

To accurately assess wounds, different management strategies have to be developed. This depends on the type and extremity of injury, which is of great importance. Sometimes, injuries of high extremity require amputation. Therefore, it becomes essential to classify wounds based on the treatment strategy that has to be employed. One such classification system is the Rank and Wakefield classification (Mackay 1995; Vidyarthi and Gupta 2003; Purcell 2016). According to the Rank and Wakefield point of view, wounds are divided into two broad categories: tidy and untidy

Table 1 Classification of the wounds according to etiology

	Type of wounds	Description	References
1.	Blunt trauma wounds	When a blunt object directly comes in contact with the body.	Simon et al. (2020)
2.	Burn injuries	When a small area of the body or other tissue is affected by burns.	Spanholtz et al. (2009)
3.	Penetrating injuries	When the skin is pierced due to the penetration of a foreign object in the body and leads to an open wound.	Kuhajda et al. (2014)
4.	Incisional wounds	When an incision/cut is made through muscle causing damage and disruption to the tissues.	Gamelli and He (2003)

Table 2 Classification of the wounds according to the Rank-Wakefield system

	Type of wounds	Description	References
1.	Tidy wounds	These are inflicted by sharp objects causing minimal contamination and tissue remains well-vascularized on the edge of the skin. Primary healing occurs with time.	Mackay (1995), Vidyarthi and Gupta (2003), Purcell (2016)
2.	Untidy wounds	Results from tearing, crushing, avulsion, vascular injury, or burns. These contain devitalized tissue and require debridement of all the devitalized tissue to create a tidy wound for the initial assessment. Healing occurs through secondary approaches.	Mackay (1995), Vidyarthi and Gupta (2003)

wounds (Table 2). This was one of the earliest and simplest modes of classification and served the purpose of guiding wound management strategies.

2.3 Classification of the Wounds According to the Duration of Wound Healing

Time plays a vital role in injury management and healing or repair of wounds. Thus, based on the time frame of the healing process, wounds can be surgically categorized as acute and chronic. These two terms are considered as references to the cause as well as the time frame of the wound recovery (Bowler et al. 2001; Moreo 2005; Morton and Phillips 2016; Powers et al. 2016; Maqsood 2018). The timeframe for this criterion to be applied and to consider a wound as chronic is approximately 6 weeks. Any wound which can be expected to heal in a shorter period can be classified as acute (Bowler et al. 2001; Moreo 2005; Korting et al. 2011; Jacobsona et al. 2017; Maqsood 2018). Chronic wounds are ones that do not proceed through the standard stages of wound healing in an appropriate and timely fashion. Chronic wounds are frequently found to stall in the inflammation phase of healing (Quirinia 2000; Alexiadou and Doupis 2012). One of the critical examinations to be performed on chronic wound patients is the vascular examination since the main driving factor for potential healing of these wounds is the proper recruitment of immune cells through the bloodstream. Chronic wounds are also characterized by an absence of balance in the production and degradation of cells in the wound healing process (Alexiadou and Doupis 2012; Morton and Phillips 2016; Powers et al. 2016) (Table 3). The differing types and subtypes of wounds, when classified according to the duration of wound healing, are described below.

Table 3 Classification of the wounds according to the duration of wound healing

	Type of wounds	Description	References
1.	**Acute wounds**	These arise out of damage to bony structures and soft tissues, which involves a trauma injury caused by environmental factors such as knife cuts, insect bites, burns, etc.	Moreo (2005), Maqsood (2018)
	Scrapes or abrasion	Skin causes friction when rubbed against rough surfaces. E.g., Skinned knees and rope burns.	Korting et al. (2011)
	Missile or velocity wounds	Deep body tissue damage caused by a high-speed gunshot.	Hamdan (2006), Lone et al. (2009)
	Contusions or avulsion	Wounds caused due to breaking of bones by a forcible strike on the body or getting pulled away from the rest of the bone. E.g., Hit by a ball or loss of nail, tooth, etc.	Broder (2011)
	Cut or crush wounds	When a sharp or heavy object falls on the body, causing a slice or cut. Road injuries are also included in this category, where damage occurs to the dermis and parts of the hypoderm.	Bowler et al. (2001), Montella et al. (2014)
	Lacerations	Tearing of soft body tissues, which can either be internal or external. E.g., Punching body and childbirth.	Pergialiotis et al. (2014)
	Radiation wounds or ulcers	Injuries caused to the underlying soft tissues by ionizing radiation. E.g., Chemotherapy.	Jacobsona et al. (2017)
2.	**Chronic wounds**	These are caused by metabolic perturbations or tissues injuries which heal slowly.	Morton and Phillips (2016), Powers et al. (2016)
	Venous/vascular ulcers	Occurs in the lower extremities in the legs also known as stasis ulcer or dermatitis. Usually seen in old age groups.	Vasudevan (2014)
	Diabetic wounds/ulcers	Due to neuropathic conditions and compromised immune system, the body is unable to prevent infection and turns small wounds into chronic.	Alexiadou and Doupis (2012)
	Pressure ulcers	Also known as bedsores and normally found in the paralytic condition. Due to the immobility of the body, blood flow is restricted in muscles and tissues.	Bhattacharya and Mishra (2015)
	Ischemic wounds	It occurs as a result of a clinical blockage of blood supply to vascular beds, resulting in glucose and oxygen shortage for cellular metabolism.	Quirinia (2000)

2.4 Classification of the Wounds According to the Integrity of the Skin

Another method of classification, that is based on the degree of damage to the integrity of the skin. This method heavily dictates wound management and healing

Table 4 Classification of the wounds according to the integrity of the skin

	Type of wounds	Description	References
1.	Open wound	These injuries occur due to skin laceration with or without tissue loss.	Bauer and Aiken (1989), John et al. (2014)
2.	Closed wound	These injuries occur without any disruption in skin integrity and the skin remains intact.	John et al. (2014)

strategy since the integrity of the skin is the key component required for proper repair and regeneration (Bauer and Aiken 1989; John et al. 2014). It is a broad and straightforward mode of classification since it does not entail many categories or subtypes. A different version of the same classification emerges when we classify wounds into superficial and deep wounds (Bauer and Aiken 1989; John et al. 2014). The major criterion is the capacity of the skin to heal itself, which depends upon the extent of damage to the skin, which can then leads us to the classification as superficial, deep dermal, or full-thickness (Table 4). When classified according to the integrity of the skin, the wounds can be divided into two groups, as described below.

2.5 Classification of the Wounds According to the Bacterial Contamination or the Degree of Contamination

It is essential to restrict the spread of injury and infection caused by the wounds, and for that, it is essential to confirm that the wounds are properly cleaned and appropriately dressed. One can surmise that a surgical wound is considered a contaminated wound when an external object has come into contact with the skin leading to a high risk of infection (Devaney and Rowell 2004; Sarabahi and Tiwari 2012; Onyekwelu et al. 2017; Gorvetzian et al. 2018; Herman and Bordoni 2020)'. The extent to which the contamination has occurred dictates the wound management strategy and the urgency to clean up the wound (Table 5). When there is a presence of wound contamination, the optimal method of management is to proceed with wound closure after an initial period of delay. Thus, to classify the condition and cleanliness of wounds, the Centers for Diseases Control and Prevention (CDC) has established four classes of the degree of contamination (Devaney and Rowell 2004; Sarabahi and Tiwari 2012; Onyekwelu et al. 2017; Gorvetzian et al. 2018; Herman and Bordoni 2020)' as described below.

Table 5 Classification of the wounds based on the degree of contamination

	Type of wounds	Description	References
1.	Clean wounds	Uninfected surgical wounds where no internal damage is seen and only skin microflora has been contaminated such as hernia repair, exploratory laparotomy.	Sarabahi and Tiwari (2012), Herman and Bordoni (2020)
2.	Clean-contaminated wounds	These wounds occur when surgical wounds with microbial flora, under an uncontrolled condition, penetrate the genitourinary tract, respiratory, and alimentary tract. E.g., Hysterectomy, lobectomy.	Margenthaler et al. (2003), Devaney and Rowell (2004)
3.	Contaminated wounds	These wounds are marked by the introduction of microflora in a previously uncontaminated part of the body due to a major break in aseptic technique or gross spillage from the intestinal tract (cholecystectomy with bile spillage or acute inflammation).	Devaney and Rowell (2004), Gorvetzian et al. (2018)
4.	Heavily contaminated wounds	These are typically old traumatic wounds in which necrotic tissues are present that involve clinical infection, such as infection including repair of a perforated bowel.	Onyekwelu et al. (2017), Gorvetzian et al. (2018)

2.6 Classification of the Accidental Wounds Based on Their Origin

A clue to the cause for any skin lesions or wound can be determined from the physical assessment of the injury. Molecular mechanisms governing skin wound healing are still not completely understood. This puts the onus on the primary level of wound assessment and classification to guide wound management strategies. Clinicians find it difficult to have a unanimous denomination of wounds, since each tissue that has to heal, or the reason that initiates the wound will dictate a distinct approach (Ikpeme et al. 2010; Abrahamian and Goldstein 2011; Iyer and Balasubramanian 2012; Okonkwo and DiPietro 2017; VanHoy et al. 2020; Schaefer and Tannan 2020; Sveen et al. 2020). The final mode of classification discussed here involves assessing the root cause or origin of the wound. The major benefit of this approach is that it expedites the initial step towards wound healing and thereby improves clinical outcomes. For this purpose, Table 6 briefly describes the classification of wounds based on the morphology and pathophysiological process for the type of damage.

Table 6 Classification of the wounds according to the origin

	Type of wounds	Description	References
1.	**Mechanical wounds**	Disruption of the skin integrity including the mucous layer, caused by any mechanical force on the body. Mechanical wounds can be divided into abraded/abrasion wounds, puncture wounds, incised wounds, cut wounds, crush wounds, torn wound, bite wound, shot wound, etc.	Sveen et al. (2020)
2.	**Chemical wounds**		
	Acid wounds	These occur due to exposure to acids causing chest pain and vomiting.	VanHoy et al. (2020)
	Base wounds	Similar to acid wounds but more toxic. Necrotic tissue becomes liquified, and lysis of cell and protein occurs.	VanHoy et al. (2020)
3.	**Wounds caused by radiation**	Tissue damage caused by radiation exposure.	Iyer and Balasubramanian (2012)
4.	**Wounds caused by thermal stress**		
	Burning wounds	These are skin injuries caused by contact with hot surfaces, flame, hot liquids, or steam.	Schaefer and Tannan (2020)
	Freezing wounds	Injuries caused by the cold temperature that leads to contraction of the blood vessels and results in thrombosis.	Sarabahi and Tiwari (2012)
5.	**Wounds caused by diseases**		
	Bone infection	Inflammation of bones by bacteria or fungus.	Ikpeme et al. (2010)
	Diabetes	Due to neuropathic conditions and compromised immune system body is unable to prevent infection and turns small wounds into chronic. This is a type of chronic wound.	Boniakowski et al. (2017), Okonkwo and DiPietro (2017)
	Gangrene	Necrosis caused by bacterial infection leading to the death of body tissue.	Tan et al. (2018)
	Immunosuppressive disorder wounds	Surgical wounds associated with the compromised immune system.	Raje and Dinakar (2015)
6.	**Surgical wounds**	An incision/cut usually made during surgery using a scalpel.	Onyekwelu et al. (2017)
7.	**Microbial infection**	Penetration of pathogens or microbes through cuts on the body, causing diseases.	Aly (1996)
8.	**Wounds caused by animals**	Wounds caused by an animal bite.	Bjornstig et al. (1991), Abrahamian and Goldstein (2011)

3 Physiology of Wound Healing

A consecutive loss of function in any anatomical structure, which leads to tissue disruption can be described as a wound. As soon as any damage is done to the tissue, organ, or body, multiple parallel and interrelated pathways are activated (Singer and Clark 1999; Gurtner et al. 2008; Eming et al. 2014; Martin and Nunan 2015; Oishi and Manabe 2018; Lim et al. 2019; Rodrigues et al. 2019). These cellular and extracellular pathways work in a coordinated manner, and their corresponding functions must be carried out in the proper sequence, at the appropriate time to achieve wound healing (Gosain and DiPietro 2004; Guo and Dipietro 2010; Bielefeld et al. 2013; Sgonc and Gruber 2013; Eming et al. 2014; Bonifant and Holloway 2019; Rodrigues et al. 2019). Once the healing is completed, these pathways are stopped in a precise order to avoid extreme reactions or delayed responses (Bayat et al. 2003; Diegelmann and Evans 2004; Rodrigues et al. 2019). Despite the intricate nature of wound healing pathways, it is noteworthy that these mechanisms that regularly take place in the human body are precisely programmed and work without complication (Guo and Dipietro 2010). Interruptions in these processes can lead to delayed wound healing and a high risk of patient mortality.

Wound healing is a common phenomenon of repair, growth, and tissue regeneration (Hunt et al. 2000; Diegelmann and Evans 2004; Broughton et al. 2006; Boateng et al. 2008; Velnar et al. 2009; Wang et al. 2018). In other words, this is a complex and dynamic biological process that requires intricate spatial and temporal synchronization of various cell types and mediators, interacting in an extremely sophisticated cascade of cellular events (Hunt et al. 2000; Gonzalez et al. 2016; Sorg et al. 2017; Kabashima et al. 2019; Rodrigues et al. 2019). During the healing process, various immune cells, such as neutrophils, monocytes, Langerhans cells (LCs), γδ T-cells, CD+ resident memory T-cells (TRM), and others are recruited at the wound site. In response to micro-environmental conditions and different cytokines, these cells maintain homeostasis upon inflammatory challenges (Hunt et al. 2000; Gurtner et al. 2008; Singh et al. 2017; Rodrigues et al. 2019). Details of these cells and chemical factors are provided in Tables 7 and 8, respectively, and also in succeeding sections. Since the process of wound repair is continuous, at the time of injury, various cellular and biological events are activated to restore the integrity of skin (Hunt et al. 2000; Gonzalez et al. 2016; Sorg et al. 2017; Kabashima et al. 2019; Rodrigues et al. 2019). Therefore, to better understand this physiological process that is happening at the wound site and nearby tissues, molecular events in wound repair are categorized into the following stages: hemostasis, inflammatory phase, angiogenesis, growth phase, re-epithelialization, and tissue maturation and remodeling (Hunt et al. 2000; Guo and Dipietro 2010; Sgonc and Gruber 2013; Eming et al. 2014; Gonzalez et al. 2016; Sorg et al. 2017; Bonifant and Holloway 2019; Rodrigues et al. 2019). These stages of wound healing proceed sequentially but also overlap with each other (Table 9 and Fig. 2).

The primary response in the process of wound healing is: contraction of the wounded blood vessels as well as platelet activation to form a fibrin clot, ultimately

Table 7 Cells involved in wound healing. The table has been reproduced with permission from Singh S. et al., 2017, Surgery (Singh et al. 2017) and Greaves N. S. et al., J. 2013, Dermatol. Sci. (Greaves et al. 2013)

Cell type	Time of action	Growth factors released	Function	References
Platelets	Immediately after injury	TGF-α, TGF-β, IL-1, lipoxins, leukotrienes, thromboxane-A_2, TNF-α, serotonin IGF-1, CTGF, VEGF, PDGF, EGF, FGF-2	• Release of inflammatory mediators (EGF, TGF-β, PDGF, FGF, serotonin, histamine, thromboxane, prostaglandins, bradykinin) • Activation of the coagulation cascade • Thrombus formation	Abe et al. (2001), Egozi et al. (2003), Workalemahu et al. (2003), Anitua et al. (2004), Sun et al. (2009), Johnston et al. (2011), Greaves et al. (2013), Singh et al. (2017)
Neutrophils	1–48 h	TGF-β, CTGF, TNF-α, IL-1	• Release of proteolytic enzymes and ROS generation • Increase vascular permeability • Phagocytosis of bacteria • Wound debridement	Egozi et al. (2003), Werner and Grose (2003), Workalemahu et al. (2003), Strid et al. (2004), Hu et al. (2010), Greaves et al. (2013), Singh et al. (2017)
Keratinocytes	8 h	TGF-β, EGF, FGF-2, VEGF, IGF-1, TNF-α, IL-1, uPA, tPA, PAI-1	• Releases inflammatory mediators • Stimulate adjacent keratinocytes • Neovascularization	Sahni and Francis (2000), Egozi et al. (2003), Workalemahu et al. (2003), Sun et al. (2009), Hu et al. (2010), Greaves et al. (2013), Singh et al. (2017)
Lymphocytes	72–120 h	IL-1, interferon-γ	• Regulates proliferative phase of wound healing • Collagen deposition	Werner and Grose (2003), Strid et al. (2004), Hu et al. (2010), Mahdavian Delavary et al. (2011), Greaves et al. (2013), Singh et al. (2017)
Fibroblasts	120 h	TGF-β, HGF, FGF-2, PDGF, VEGF, CTGF,	• Synthesis of granulation tissue • Produces ECM components	Abe et al. (2001), Yang et al. (2005), Conway et al. (2006), Giannouli

(continued)

Table 7 (continued)

Cell type	Time of action	Growth factors released	Function	References
		IGF-1, Ang-1, Ang-2, uPA	• Collagen synthesis • Release of inflammatory mediators and various proteases	and Kletsas (2006), Sun et al. (2009), Greaves et al. (2013), Singh et al. (2017)

CTGF connective tissue growth factor; *ECM* extracellular matrix; EGF: Epidermal growth factor; *FGF-2* Fibroblast growth factor-2; *HGF* hepatocyte growth factor; *IGF-1* insulin-like growth factor; *IL* Interleukin; *PAI* plasminogen activator inhibitor; *PDGF* platelet-derived growth factor; *TGF-α* transforming growth factor-α; *TGF-β* transforming growth factor-β; *TNF-α* tumor necrosis factor-α; *tPA* tissue plasminogen activator; *ROS* reactive oxygen species; *uPA* urokinase plasminogen activator; *VEGF* vascular endothelial growth factor

stopping the bleeding (Clark 2003; Geer and Andreadis 2003; Cogle et al. 2004; Rodrigues et al. 2019). Immediately after clot formation, the injured tissues release growth factors and pro-inflammatory cytokines (see Table 8 for detail). As soon as the bleeding is controlled, various inflammatory cells, for instance, neutrophils, monocytes, and macrophages, are recruited at the wound site to promote the inflammatory phase. Additionally, the adaptive immune system also gets activated to fight against a variety of self and foreign antigens (Park and Barbul 2004; Brown and Watson 2011; Davies et al. 2013a, b; Hoeffel and Ginhoux 2018; Larouche et al. 2018; Rodrigues et al. 2019). In the next phase, closely aligned with the inflammation phase, new blood vessel formation ensues through angiogenesis (Rodrigues et al. 2019). This involves the activity of neuromas cell types within the perivascular space. Here, along with the proliferation of the endothelial cells, circulating progenitor cells from the bone marrow and pericytes within the basal lamina also participate in new blood vessel formation within the perivascular space (Asahara et al. 1997; Ceradini et al. 2004; Armulik et al. 2011; Ansell and Izeta 2015; Kosaraju et al. 2016; Zhan et al. 2018; Rodrigues et al. 2019). It is then followed by fibroblast migration and proliferation, synthesis of the matrix proteins, ECM formation, keratinocyte proliferation, and differentiation, regeneration of hair follicles, etc. during the growth and proliferative phase of wound healing (Martin 1997; Werner et al. 2007; Donati et al. 2017; Rodrigues et al. 2019). Finally, the reorganization and remodeling of ECM, as well as the rearrangement of granulation tissue to scar tissue, completes the wound healing process, and synthesis as well as cross-linking of collagen provide stability to the healing tissue. In this section, we discuss each of these processes in detail to understand the physiology of wound healing (Table 9).

Table 8 Growth factors involved in wound healing. The table has been reproduced with permission from Singh S. et al., 2017, Surgery. (Singh et al. 2017) and Greaves N. S. et al., J. 2013, Dermatol. Sci. (Greaves et al. 2013)

Factor	Released from	Phase of wound healing	Action	Physiological effects during wound healing	References
TGF-α	Platelets, macrophages	Angiogenesis	• Granulation tissue formation • Stimulates proliferation of fibroblasts and epithelial cell	• Leucocyte recruitment	Abe et al. (2001), Egozi et al. (2003), Workalemahu et al. (2003), Mahdavian Delavary et al. (2011), Greaves et al. (2013), Singh et al. (2017)
TGF-β	Platelets, neutrophils, macrophages, fibroblasts, keratinocyte, mast cell	Fibroplasia, angiogenesis	• Chemotaxis • Trans-differentiation of fibroblasts • Stimulation of angiogenesis through collagen matrix construction • Wound contraction • MMP stimulation and release of growth factors	• Leucocyte recruitment	Abe et al. (2001), Workalemahu et al. (2003), Yang et al. (2005), Sun et al. (2009), Mahdavian Delavary et al. (2011), Greaves et al. (2013), Singh et al. (2017)
EGF	Platelets Macrophages	Fibroplasia	• Stimulates proliferation of different types of cells	• Re-epithelialization	Schultz et al. (1991), Johnston et al. (2011), Greaves et al. (2013), Singh et al. (2017)
HGF	Fibroblasts	Angiogenesis	• Accelerates healing and prevents fibrosis • Higher expression of HGF-NK2	• Re-epithelialization • Leucocyte recruitment	Kankuri et al. (2005), Conway et al. (2006), Greaves et al. (2013), Singh et al. (2017)
FGF-2	Platelet, macrophage Keratinocyte, fibroblast, endothelial cell, Fibrocyte	Fibroplasia, angiogenesis	• Helps in cell proliferation and migration • Formation of granulation tissue and skin repair	• Endothelial cell proliferation • ECM regulation via MMP-1 up-regulation	Anitua et al. (2004), Giannouli and Kletsas (2006), Xie et al. (2008), Chrissouli et al. (2010), Greaves et al. (2013), Singh et al. (2017)

(continued)

Table 8 (continued)

Factor	Released from	Phase of wound healing	Action	Physiological effects during wound healing	References
PDGF	Platelets, fibroblasts Endothelial cells, macrophages	Fibroplasia	• Chemotaxis • Fibroblast proliferation • Collagen deposition	• Leucocyte recruitment	Anitua et al. (2004), Chrissouli et al. (2010), Mahdavian Delavary et al. (2011), Greaves et al. (2013), Singh et al. (2017)
VEGF	Platelets, fibroblasts Endothelial cells, macrophages, keratinocyte, Myofibroblast, Fibrocyte, mast cell	Angiogenesis	• Chemotaxis • Fibroblast proliferation • Collagen deposition	• Vascular permeability • Endothelial cell migration and proliferation	Gaudry et al. (1997), Sahni and Francis (2000), Ohtani et al. (2007), Greaves et al. (2013), Singh et al. (2017)
CTGF	Platelet, neutrophil Monocyte, γδ + −T-cell, fibroblast	Fibroplasia, angiogenesis	• Inhibit advanced granulation tissue formation	• Scarring	Werner and Grose (2003), Workalemahu et al. (2003), Mahdavian Delavary et al. (2011), Greaves et al. (2013), Singh et al. (2017)
IGF-1	Platelet, fibroblast, keratinocyte, DETC, BM-MSC	Fibroplasia	• Re-epithelialization and granulation tissue formation of epidermal tissue • Stimulate GH secretion	• Keratinocyte pro-survival	Gillitzer and Goebeler (2001), Anitua et al. (2004), Sharp et al. (2005), Mahdavian Delavary et al. (2011), Greaves et al. (2013), Singh et al. (2017)
Ang-1	Fibroblast, Myofibroblast	Angiogenesis	• Determines the fate of blood vessel formation • Express mRNA	• Endothelial cell proliferation	Suri et al. (1998), Papapetropoulos et al. (2000), Kumar et al. (2009), Greaves et al. (2013), Singh et al. (2017)
Ang-2	Fibroblast, Myofibroblast	Angiogenesis	• Reduce VEGF in wounds	• Antagonist to Ang-1	Suri et al. (1998), Papapetropoulos et al. (2000), Kumar et al. (2009), Staton et al.

					(2010), Greaves et al. (2013), Singh et al. (2017)
Serotonin	Platelets	Fibroplasia, apoptosis	• Vasoconstriction • Platelet aggregation • Chemotaxis • Increase vascular permeability	• Cellular proliferation	Greaves et al. (2013), Singh et al. (2017), Sadiq et al. (2018)
TNF-α	Platelets, neutrophils, monocyte, macrophage, keratinocyte, mast cell	Fibroplasia	• Chemotaxis • Nitric oxide release • Activation of other growth factors	• Expression of growth factors • Leucocyte chemotaxis • ECM degradation • Keratinocyte migration	Werner and Grose (2003), Strid et al. (2004), Strid et al. (2009), Mahdavian Delavary et al. (2011), Greaves et al. (2013), Singh et al. (2017)
PGE_2	Keratinocytes, macrophages, endothelial cells	Fibroplasia	• Vasodilation • Platelet disaggregation • Increased vascular permeability • Pain • Fever	• Inhibit fibroblast migration • Inhibit FPCL contraction	Sandulache et al. (2006), Greaves et al. (2013), Singh et al. (2017)
Thromboxane-A_2	Platelets	Fibroplasia	• Platelet aggregation • Vasoconstriction	• Enhances aggregation of blood thrombocytes	Greaves et al. (2013), Singh et al. (2017), Rodrigues et al. (2019)
Leukotrienes	Platelets, leukocytes	Fibroplasia, angiogenesis	• Amplified vascular permeability • Chemotaxis • Leukocyte adhesion • Chemotaxis (neutrophils)	• Keratinocyte migration.	Greaves et al. (2013), Singh et al. (2017), Luo et al. (2017)
IL-1	Platelets, neutrophil Macrophage, monocyte, keratinocyte, endothelial cells, lymphocytes	Fibroplasia	• Chemotaxis	• Leucocyte chemotaxis • Expression of growth factors • ECM degradation	Werner and Grose (2003), Strid et al. (2004), Hu et al. (2010), Greaves et al. (2013), Singh et al. (2017)

(continued)

Table 8 (continued)

Factor	Released from	Phase of wound healing	Action	Physiological effects during wound healing	References
Lipoxins	Platelets, leukocytes	Fibroplasia	• Dampen inflammatory response • Inhibit chemotaxis (neutrophils)	• 12/15-lipoxygenase are expressed in epithelial cells	Gronert (2005), Greaves et al. (2013), Singh et al. (2017)
Interferon-γ	Fibroblasts, lymphocytes	Angiogenesis	• Macrophage maturation • Nitric oxide release	• Enhance vascular endothelial growth factor mRNA expression	Ishida et al. (2004), Greaves et al. (2013), Singh et al. (2017)
MMP-2	Dermal fibroblast	Fibroplasia, angiogenesis	• Inhibitors help in regulating extracellular matrix degradation	• ECM/collagen degradation enables endothelial cell migration	Toriseva and Kahari (2009), Greaves et al.(2013), Singh et al. (2017), Yen et al. (2018)
uPA	Macrophage, keratinocyte, endothelial cell, monocyte, fibroblast	Fibroplasia, angiogenesis	• Induces matrix proteolysis	• Fibrin dissolution and ECM degradation activation of growth factors and MMPs	Madhyastha et al. (2008), Toriseva and Kahari (2009), Greaves et al. (2013), Singh et al. (2017), Yen et al. (2018)
tPA	Keratinocyte	Fibroplasia, angiogenesis	• Synaptic plasticity and remodeling	• Dissolution of fibrin • Degradation of ECM • Activation of MMPs and various growth factors	Lund et al. (2006), Toriseva and Kahari (2009), Greaves et al. (2013), Singh et al. (2017), Yen et al. (2018)
PAI-1	Migrating epidermal keratinocyte, fibroblast	Fibroplasia, angiogenesis	• Soluble inhibitor of proteolysis • Matrix-bound regulator for cell migration.	• PA (uPA, tPA) activity regulator • Keratinocyte migration	Schafer et al. (1994), Ghosh and Vaughan (2012), Greaves et al. (2013, Singh et al. (2017)

Ang Angiopoietin; *BM-MSC* bone marrow mesenchymal stem cell; *CTGF* connective tissue growth factor; *DETC* dendritic epidermal T-cell; *ECM* extracellular matrix; *EGF* epidermal growth factor; *FGF-2* Fibroblast growth factor-2; *GH* growth hormone; *HGF* hepatocyte growth factor; *IGF-1* insulin-like growth factor; *IL* Interleukin; *MMP* matrix metalloproteinases; *MMP-2* matrix metalloproteinase-2; *PA* plasminogen activator; *PAI* plasminogen activator inhibitor; *PDGF* platelet-derived growth factor; *PGE2* Prostaglandin E2; *TGF-α* transforming growth factor-α; *TGF-β* transforming growth factor-β; *TNF-α* tumor necrosis factor-α; *tPA* tissue plasminogen activator; *uPA* urokinase plasminogen activator; *VEGF* vascular endothelial growth factor

Table 9 Major stages in the physiological process of wound healing

Phase	Main process	Events during the process	Time	References
Hemostasis	• Vasoconstriction • Platelet plug formation (primary hemostasis) • Coagulation and reinforcement of the platelet plug	• Vascular contraction • Platelet aggregation and degranulation • Thrombus formation	Immediately after injury	Mathieu et al. (2006), Guo and Dipietro (2010), Mahdavian Delavary et al. (2011), Bielefeld et al. (2013), Sun et al. (2014), Rodrigues et al. (2019)
Inflammatory phase	• Release of growth factors and cytokines by platelets, immune cells, and disrupted matrix • Invasion of inflammatory cells (neutrophils, monocytes, macrophages)	• Vascular exudation • Neutrophil infiltration • Monocyte conversion to macrophage • Matrix enrichment in proteoglycans • Lymphocyte infiltration	Day 0–3	Mathieu et al. (2006), Guo and Dipietro (2010), Mahdavian Delavary et al. (2011), Bielefeld et al. (2013), Sun et al. (2014), Rodrigues et al. (2019)
Growth and proliferative phase	• Granulation tissue formation and neovascularization • Formation of endothelial cells and new vessel • Pericytes in neovascularization and wound healing • Circulating progenitor cells in neovascularization and wound healing • Regeneration of hair follicles	• Re-epithelialization • Angiogenesis • Fibroblast infiltration and proliferation • Collagen formation • ECM formation	Day 3–15	Mathieu et al. (2006), Guo and Dipietro (2010), Mahdavian Delavary et al. (2011), Bielefeld et al. (2013), Sun et al. (2014), Rodrigues et al. (2019)
Tissue remodeling	• ECM reorganization and remodeling • Myofibroblast formation • Contraction of the wound • Cell apoptosis	• Vascular maturation and regression • Conversion of fibroblast to fibrocyte • Collagen degradation and formation	Day 15–Month to years	Mathieu et al. (2006), Guo and Dipietro (2010), Mahdavian Delavary et al. (2011), Bielefeld et al. (2013), Sun et al. (2014), Rodrigues et al. (2019)

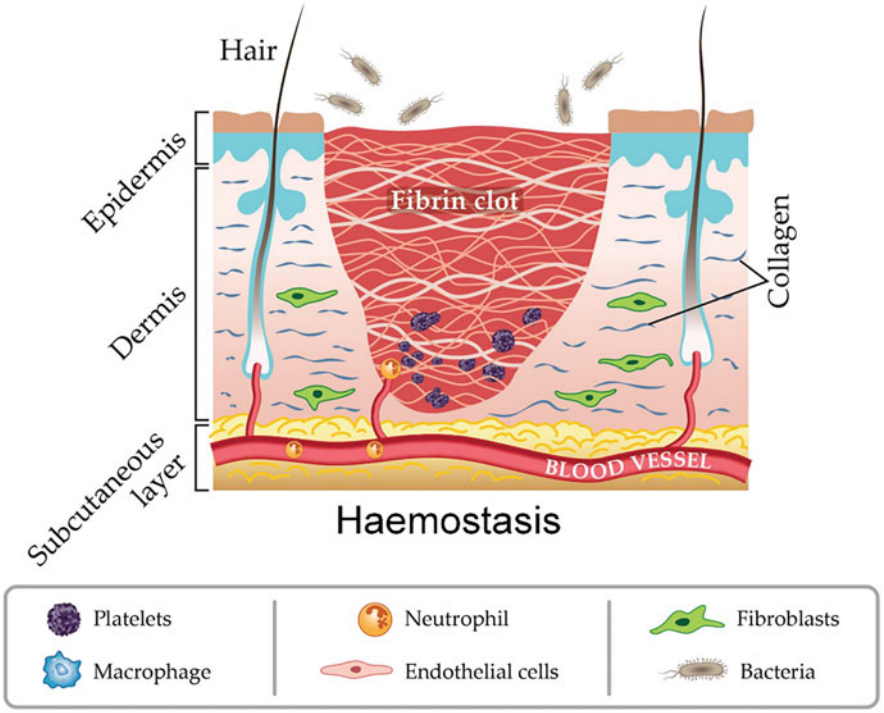

Fig. 2 Hemostasis in wound healing. This is the first phase of wound healing, which occurs as soon as any damage or injury is done on any tissue or organ. In response to the injury, the outermost layer of the skin works towards preventing excess loss of blood in a process known as vasoconstriction. Thereafter, the platelets are forced into action, where they release essential factors and drive the platelet plug formation. The final part of hemostasis stage involves the reinforcement of the platelet plug through the coagulation process. The figure is adapted with permission from Negut, I. et al. 2018, Molecules (Negut et al. 2018)

3.1 Coagulation and Hemostasis

This is the first phase in the process of wound healing, which occurs as soon as any damage or injury is done on any tissue or organ (Fig. 2, Table 9). This event occurs on a micro- or macro-vascular scale where various cellular responses promote blood clotting and prevent exsanguination or blood loss at the site of injury (Mathieu et al. 2006; Guo and Dipietro 2010; Mahdavian Delavary et al. 2011; Bielefeld et al. 2013; Singh et al. 2017; Rodrigues et al. 2019). The key point of this mechanism is (i) to prevent exsanguination in order to protect the vascular system and keep the organs unharmed during the time of injury, and (ii) to provide a scaffold for migrating cells essential for complete healing (Robson et al. 2001; Velnar et al. 2009; Guo and Dipietro 2010; Bielefeld et al. 2013; Smith et al. 2015; Singh et al. 2017; Negut et al. 2018; Rodrigues et al. 2019).