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# The Wound Healing Process: an Overview of the Cellular and Molecular Mechanisms

T Velnar[1,2], T Bailey[2] and V Smrkolj[3]

[1]Department of Neurosurgery, University Medical Centre Maribor, Maribor, Slovenia; [2]Cranfield Health, Cranfield University, Cranfield, UK; [3]Clinical Department of Traumatology, University Medical Centre Ljubljana, Ljubljana, Slovenia

**Wound healing remains a challenging clinical problem and correct, efficient wound management is essential. Much effort has been focused on wound care with an emphasis on new therapeutic approaches and the development of technologies for acute and chronic wound management. Wound healing involves multiple cell populations, the extracellular matrix and the action of soluble mediators such as growth factors and cytokines. Although the process of healing is continuous, it may be arbitrarily divided into four phases: (i) coagulation and haemostasis; (ii) inflammation; (iii) proliferation; and (iv) wound remodelling with scar tissue formation. The correct approach to wound management may effectively influence the clinical outcome. This review discusses wound classification, the physiology of the wound healing process and the methods used in wound management.**

KEY WORDS: Wounds; Wound healing; Cell proliferation; Inflammation; Wound remodelling

## Introduction

In everyday pathology, wounds remain a challenging clinical problem, with early and late complications presenting a frequent cause of morbidity and mortality.[1,2] In an attempt to reduce the wound burden, much effort has focused on understanding the physiology of healing and wound care with an emphasis on new therapeutic approaches and the continuing development of technologies for acute and long-term wound management.[3,4]

The immense social and economic impact of wounds worldwide is a consequence of their high rate of occurrence in general and their increasing frequency in the ageing population. In addition to a high number of acute wounds, there are also a large number of chronic, hard-to-heal wounds associated with diseases and abnormalities that directly or indirectly culminate in damage of the cutaneous coverage, including arterial, venous, diabetic and pressure ulcers. The prevalence of these chronic wounds increases with age. For example, it has been estimated that chronic wounds affect 120 per 100 000 people aged between 45 and 65 years and rises to 800 per 100 000 people > 75 years of

age.[1,3] Furthermore, due to the complications that accompany acute wounds, when their healing does not progress in a timely and orderly manner, they can convert into chronic wounds, which are more difficult to manage.[1]

## Wounds and wound types

A wound is defined as damage or disruption to the normal anatomical structure and function.[3] This can range from a simple break in the epithelial integrity of the skin or it can be deeper, extending into subcutaneous tissue with damage to other structures such as tendons, muscles, vessels, nerves, parenchymal organs and even bone.[2]

Wounds can arise from pathological processes that begin externally or internally within the involved organ. They can have an accidental or intentional aetiology or they can be the result of a disease process. Wounding, irrespective of the cause and whatever the form, damages the tissue and disrupts the local environment within it. A physiological response to the noxious factor results in bleeding, vessel contraction with coagulation, activation of complement and an inflammatory response.[5 – 7] Normal wound healing is a dynamic and complex process involving a series of co-ordinated events, including bleeding, coagulation, initiation of an acute inflammatory response to the initial injury, regeneration, migration and proliferation of connective tissue and parenchyma cells, as well as synthesis of extracellular matrix proteins, remodelling of new parenchyma and connective tissue and collagen deposition.[8,9] Finally, increasing the wound strength takes place in an ordered manner and culminates in the repair of severed tissues.[8 – 10]

Wound healing begins at the moment of injury and involves both resident and migratory cell populations, extracellular matrix and the action of soluble mediators. The mechanisms underlying the processes described above involve: (i) inflammatory mediators and growth factors; (ii) cell–cell and cell–extracellular matrix interactions that govern cell proliferation, migration and differentiation; (iii) events involved with epithelialization, fibroplasia and angiogenesis; (iv) wound contraction; and (v) remodelling. These mechanisms are initiated at the time of physical injury and proceed continuously throughout the repair process.[3,8,11 – 14]

Despite the fact that the processes of repair begin immediately after an injury in all tissues and that all wounds go through similar phases of healing, specialized tissues such as liver, skeletal tissue and the eye have distinctive forms of regeneration and repair and follow separate pathways.[14 – 17] Additionally, there are differences between tissues in terms of the time required to complete regeneration. Wound healing time can be diverse and some wounds may take up to a year or more to heal completely.[18 – 20] A completely healed wound is defined as one that has been returned to a normal anatomical structure, function and appearance of the tissue within a reasonable period of time. Most wounds are usually the result of simple injuries; however, some wounds do not heal in a timely and orderly manner. Multiple systemic and local factors may slow the course of wound healing by causing disturbances in the finely balanced repair processes, resulting in chronic, non-healing wounds.[3,10]

## Classification of wounds

Wounds can be classified according to various criteria.[3] Time is an important factor in injury management and wound repair. Thus, wounds can be clinically categorized

as acute and chronic according to their time frame of healing.[3,5,6]

## ACUTE WOUNDS

Wounds that repair themselves and that proceed normally by following a timely and orderly healing pathway, with the end result of both functional and anatomical restoration, are classified as acute wounds. The time course of healing usually ranges from 5 to 10 days, or within 30 days. Acute wounds can be acquired as a result of traumatic loss of tissue or a surgical procedure.[3,5] For example, an operation to remove a soft tissue tumour located in the skin and underlying parenchyma can sometimes result in a large albeit non-contaminated wound that cannot be healed by primary intention, due to the large defect within the tissue. Traumatic wounds are also frequently encountered. They may involve only the soft tissue or they might be associated with bone fractures. These combined injuries have been classified by the classification system of the AO Foundation (Arbeitsgemeinschaft fuer Osteosynthesefragen/Association for the Study of Internal Fixation),[21] which is one of the most comprehensive and widely used. Included within this classification system are closed and open fractures with the assessment of skin, muscle, tendon and neurovascular injuries.[21] A benefit of the AO Foundation's classification system is that the extent of damage to muscles and tendons is taken into account, as it determines the prognosis of the injured limb.[6 – 8]

## CHRONIC WOUNDS

Chronic wounds are those that fail to progress through the normal stages of healing and they cannot be repaired in an orderly and timely manner.[3,4] The healing process is incomplete and disturbed by various factors, which prolong one or more stages in the phases of haemostasis, inflammation, proliferation or remodelling. These factors include infection, tissue hypoxia, necrosis, exudate and excess levels of inflammatory cytokines.[22] A continuous state of inflammation in the wound creates a cascade of tissue responses that together perpetuate a non-healing state. Because the healing then proceeds in an unco-ordinated manner, functional and anatomical outcomes are poor and these wounds frequently relapse.[3,23] Chronic wounds may result from various causes, including naturopathic, pressure, arterial and venous insufficiency, burns and vasculitis.[20,23]

## COMPLICATED WOUNDS

A complicated wound is a special entity and is defined as a combination of an infection and a tissue defect.[6] Infection poses a constant threat to the wound. The cause of the defect, in contrast, evolves due to the traumatic or post-infectious aetiology, or a wide tissue resection (e.g. in tumour management). Every wound is contaminated irrespective of the cause, size, location and management. Whether or not a manifest infection develops depends on the virulence, number and type of micro-organisms, as well as on the local blood supply and the patient's inherent resistance. Typical characteristics of infection are the five signs and symptoms that have been well documented: redness, heat, pain, oedema and loss or limited function in the affected part. The frequency of wound infections depends on the type or surgical technique and the location of the wound.[6,23,24]

Other criteria taken into account during wound classification include aetiology, degree of contamination, morphological characteristics and communication with hollow or solid organs.[3,6,20] Aetiology

classifies wounds according to the trigger factor into contusions, abrasions, avulsions, lacerations, cuts, stab wounds, crush wounds, shot wounds and burns.[3,5,6,11] According to the degree of contamination, wounds are classified into three groups as follows: (i) aseptic wounds (bone and joint operations); (ii) contaminated wounds (abdominal and lung operations); and (iii) septic wounds (abscesses, bowel operations, etc).[11,20,22] Wounds may also be referred to as closed, where the underlying tissue has been traumatized but the skin has not been severed, or as open, where the skin layer has been damaged with the underlying tissue exposed.[6,20,23,24]

## The wound healing process

Wounding and wound healing take place in all tissues and organs of the body. Many of these repair processes are common to all tissues. Although the process of healing is continuous, it is arbitrarily divided into different phases in order to aid understanding of the physiological processes that are taking place in the wound and surrounding tissue.[19] Healing is a complex process involving co-ordinated interactions between diverse immunological and biological systems. It involves a cascade of carefully and precisely regulated steps and events that correlate with the appearance of various cell types in the wound bed during distinct phases of the healing process.[24 – 27]

Separate parts of a wound may be at different stages of healing at any one time.[6,19,20,25] Timing and interactions between the components taking part in the wound healing process differ for acute and chronic wounds, although the main phases remain the same.[3,27,28] The various processes of acute tissue repair, which are triggered by tissue injury, may be united into a sequence of four time-dependent phases: (i) coagulation and haemostasis, beginning immediately after injury; (ii) inflammation, which begins shortly thereafter; (iii) proliferation, which starts within days of the injury and encompasses the major healing processes; and (iv) wound remodelling, in which scar tissue formation takes place, and which may last up to a year or more.[22 – 26]

### COAGULATION AND HAEMOSTASIS PHASE

Immediately after injury, coagulation and haemostasis take place in the wound.[11 – 13] The principal aim of these mechanisms is to prevent exsanguination. It is a way to protect the vascular system, keeping it intact, so that the function of the vital organs remains unharmed despite the injury. A second aim is a long-term one, which is to provide a matrix for invading cells that are needed in the later phases of healing.[3,12 – 14] A dynamic balance between endothelial cells, thrombocytes, coagulation, and fibrinolysis regulates haemostasis and determines the amount of fibrin deposited at the wound site, thereby influencing the progress of the reparative processes.[13,14]

Noxious insult causes microvascular injury and extravasation of blood into the wound.[10,11,13] Owing to the neuronal reflex mechanism, injured vessels constrict rapidly due to contraction of vascular smooth muscle cells in the circular muscle layer. The contraction is strong enough to prevent bleeding from an arteriole with a diameter of 0.5 cm. The process is, however, only effective in transversally interrupted vessels and may cause complete cessation of blood leakage. In contrast, in longitudinally severed arterioles it increases the gap.[13,14] Reflex vasoconstriction can temporarily reduce or even stop the amount of bleeding. The vascular smooth muscle tone is, however, only useful for a few minutes until

hypoxia and acidosis in the wound wall cause their passive relaxation, and bleeding resumes. Were it not for the formation of an insoluble fibrin plug, the haemostatic mechanisms alone would be ineffective over the longer term.[3,10]

Together with haemostatic events, the coagulation cascade is activated through extrinsic and intrinsic pathways, leading to platelet aggregation and clot formation in order to limit blood loss.[12,13] As blood spills into the site of injury, the blood components and platelets come in contact with exposed collagen and other extracellular matrix components. This contact triggers the release of clotting factors from the platelets and the formation of a blood clot, composed of fibronectin, fibrin, vitronectin and thrombospondin.[3,13,14,17] The blood clot and platelets trapped within it are not only important for haemostasis, as the clot also provides a provisional matrix for cell migration in the subsequent phases of the haemostatic and inflammatory phases. The cytoplasm of platelets contains α-granules filled with growth factors and cytokines, such as platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor and insulin-like growth factors.[14] These molecules act as promoters in the wound healing cascade by activating and attracting neutrophils and, later, macrophages, endothelial cells and fibroblasts.[11,14] Platelets also contain vasoactive amines, such as serotonin, that are stored in dense bodies and cause vasodilation and increased vascular permeability, leading to fluid extravasation in the tissue that results in oedema which, in turn, potentiates itself during the following inflammatory phase.[14,19] Eicosanoids and other products of arachidonic acid metabolism are released after injury to cell membranes and have potent biological functions in the immediate inflammatory response.[3,10,14,15]

## INFLAMMATORY PHASE

The humoral and cellular inflammatory phase follows next, with the aim of establishing an immune barrier against invading micro-organisms. It is divided into two separate phases, an early inflammatory phase and a late inflammatory phase.[16]

### *Early inflammatory phase*

Starting during the late phase of coagulation and shortly thereafter, the early inflammatory response has many functions. It activates the complement cascade and initiates molecular events, leading to infiltration of the wound site by neutrophils, whose main function is to prevent infection.[11] The neutrophils start with the critical task of phagocytosis in order to destroy and remove bacteria, foreign particles and damaged tissue. Phagocytotic activity is crucial for the subsequent processes, because acute wounds that have a bacterial imbalance will not heal.[3,7,16]

The neutrophils begin to be attracted to the wound site within 24 – 36 h of injury by various chemoattractive agents, including TGF-β, complement components such as C3a and C5a, and formylmethionyl peptides produced by bacteria and platelet products.[3] Due to alterations in the regulation of surface adhesion molecules, neutrophils become sticky and, through a process of margination, begin to adhere to the endothelial cells in the post-capillary venules surrounding the wound.[14,16] Then, the neutrophils roll along the surface of the endothelium being pushed forward by the blood flow. These adhesions and rolling mechanisms are mediated by selectin-dependent interactions and are classified as weak attachments.[16,17] Chemokines secreted by endothelial cells

rapidly activate a stronger adhesion system, which is mediated by integrins.[17,18] Cells stop rolling and migrate out of the venules, squeezing between the endothelial cells by a process known as diapedesis.[16 – 18] The subsequent migration now depends on chemokines and other chemotactic agents. Once in the wound environment, neutrophils phagocytose foreign material and bacteria, destroying them by releasing proteolytic enzymes and oxygen-derived free radical species.[17 – 19,25]

Neutrophil activity gradually changes within a few days of wounding, once all the contaminating bacteria have been removed.[16,17] Upon completing the task, the neutrophils must be eliminated from the wound prior to progression to the next phase of healing. Redundant cells are disposed of by extrusion to the wound surface as slough and by apoptosis, allowing elimination of the entire neutrophil population without tissue damage or potentiating the inflammatory response.[16,26] The cell remnants and apoptotic bodies are then phagocytosed by macrophages.[3,25 – 28]

***Late inflammatory phase***

As part of the late inflammatory phase, 48 – 72 h after injury, macrophages appear in the wound and continue the process of phagocytosis.[16,29 – 34] These cells are originally blood monocytes that undergo phenotypic changes on arrival into the wound to become tissue macrophages. Attracted to the wound site by a myriad of chemoattractive agents, including clotting factors, complement components, cytokines such as PDGF, TGF-β, leukotriene $B_4$ and platelet factor IV, as well as elastin and collagen breakdown products, macrophages have a longer lifespan than neutrophils and continue to work at a lower pH.[35,36] These cells are fundamental for the late stages of the inflammatory response, acting as key regulatory cells and providing an abundant reservoir of potent tissue growth factors, particularly TGF-β, as well as other mediators (TGF-α, heparin binding epidermal growth factor, fibroblast growth factor [FGF], collagenase), activating keratinocytes, fibroblasts and endothelial cells.[25 – 28,33 – 35] Clearly the depletion of monocytes and macrophages from the wound causes severe healing disturbances due to poor wound debridement, delayed fibroblast proliferation and maturation, as well as delayed angiogenesis, resulting in inadequate fibrosis and a more weakly repaired wound.[17,31 – 33]

The last cells to enter the wound site in the late inflammatory phase are lymphocytes, attracted 72 h after injury by the action of interleukin-1 (IL-1), complement components and immunoglobulin G (IgG) breakdown products.[16,25,26] The IL-1 plays an important role in collagenase regulation, which is later needed for collagen remodelling, production of extracellular matrix components and their degradation.[16,29,30]

## PROLIFERATIVE PHASE

When ongoing injury has ceased, haemostasis has been achieved and an immune response successfully set in place, the acute wound shifts toward tissue repair.[16 – 19] The proliferative phase starts on the third day after wounding and lasts for about 2 weeks thereafter. It is characterized by fibroblast migration and deposition of newly synthesized extracellular matrix, acting as a replacement for the provisional network composed of fibrin and fibronectin. At the macroscopic level, this phase of wound healing can be seen as an abundant formation of granulation tissue. The diverse processes that take place in the proliferative phase are briefly discussed below.[27 – 29]

***Fibroblast migration***

Following injury, fibroblasts and myofibroblasts in the surrounding tissue are stimulated to proliferate for the first 3 days.[33] They then migrate into the wound, being attracted by factors such as TGF-β and PDGF, that are released by inflammatory cells and platelets.[37] Fibroblasts first appear in the wound on the third day after injury and their accumulation requires phenotypic modulation. Once in the wound, they proliferate profusely and produce the matrix proteins hyaluronan, fibronectin, proteoglycans and type 1 and type 3 procollagen. All of their products are deposited in the local milieu.[3,33,35]

By the end of the first week, abundant extracellular matrix accumulates, which further supports cell migration and is essential for the repair process.[30,35] Now, fibroblasts change to their myofibroblast phenotype. At this stage, they contain thick actin bundles below the plasma membrane and actively extend pseudopodia, attaching to fibronectin and collagen in the extracellular matrix.[35,37] Wound contraction, which is an important event in the reparative process that helps to approximate the wound edges, then takes place as these cell extensions retract. Having accomplished this task, redundant fibroblasts are eliminated by apoptosis.[38 – 40]

***Collagen synthesis***

Collagens are an important component in all phases of wound healing. Synthesized by fibroblasts, they impart integrity and strength to all tissues and play a key role, especially in the proliferative and remodelling phases of repair.[39 – 41] Collagens act as a foundation for the intracellular matrix formation within the wound. Unwounded dermis contains 80% type 1 and 25% type 3 collagen, whereas wound granulation tissue expresses 40% type 3 collagen.[3]

***Angiogenesis and granulation tissue formation***

Modelling and establishment of new blood vessels is critical in wound healing and takes place concurrently during all phases of the reparative process. In addition to attracting neutrophils and macrophages, numerous angiogenic factors secreted during the haemostatic phase promote angiogenesis.[38,42,43]

Resident endothelial cells are responsive to a number of angiogenic factors, including FGF, vascular endothelial growth factor (VEGF), PDGF, angiogenin, TGF-α and TGF-β. A fine balance is kept by the action of inhibitory factors, such as angiostatin and steroids.[44 – 48] Inhibitory and stimulatory agents act on proliferating endothelial cells directly as well as indirectly, by activating mitosis, promoting locomotion and by stimulating the host cells to release endothelial growth factors.[49,50] Under hypoxic conditions, molecules are secreted from the surrounding tissue, promoting proliferation and growth of endothelial cells. In response, a four-step process takes place: (i) production of proteases by endothelial cells for degradation of the basal lamina in the parent vessel in order to crawl through the extracellular matrix; (ii) chemotaxis; (iii) proliferation; and (iv) remodelling and differentiation. FGF and VEGF play central regulatory roles in all of the processes.[43,45,46,49 – 51] Initially, there is no vascular supply in the wound centre, so viable tissue, which is limited to the wound margins, is perfused by uninjured vessels and by diffusion through undamaged interstitium.[3,26,52] Capillary sprouts from the surrounding edges invade the wound clot and, within a few days, a microvascular

network composed of many new capillaries is formed.

Chemotaxis is the ability of cells to move along a chemical gradient.[53] This biochemical mechanism enables cells to reply properly to environmental stimuli that determine proliferation, differentiation and migration. Chemotactic agents act on cell surface receptors to direct the cell migration that is involved in angiogenesis during wound healing.[54,55] The contributing factors act as mediators for neovascularization and vessel repair at the injury site. They are also important modulators of cell growth and differentiation, and they include endothelial cell growth factor, TGF-α, VEGF, angiopoietin-1, fibrin and lipid growth factors.[56 – 58] Migration is the consequence of chemotactic activity and is necessary for angiogenesis.[59,60] As a complex process that involves co-ordinated changes in cytoskeletal organization, signal transduction and cell adhesion, migration is dependent on the actin-rich network beneath the plasma membrane and is regulated by physical and chemical factors in the vascular system.[59] Regulation is achieved by three mechanisms: (i) chemotaxis (migration towards the concentration gradient of the chemoattractive substance); (ii) mechanotaxis (migration induced by mechanical forces); and (iii) haptotaxis (migration in response to a gradient of immobilized ligands).[59,60]

Cellular motility requires three distinct actions: protrusion at the cell front; adhesion, to attach the actin cytoskeleton to the substratum; and finally traction, propelling the trailing cytoplasm forward.[61 – 63]

***Protrusion***

With three types of interconnected filaments, the cytoskeleton is anchored at cell–cell junctions and cell–extracellular matrix adhesions, providing mechanical support for the cell.[59] The actin network is well known for its dynamic reorganization, it acts as a mechano-effector and it is important for co-ordinating cell migration. During the first step of locomotion, actin polymerization takes place at the leading edge, determined by the highest concentration of chemoattractive substance, pushing the plasma membrane outward. A protruding structure forms, in the case of the endothelial cell these are known as filopodia, and they are filled with filamentous actin.[64] Unidirectional movement of the cell is maintained through the action of a cyclic assembly and disassembly of actin filaments in front of and well behind the leading edge, respectively.[63,65] Multiple signalling pathways and regulatory proteins control actin dynamics and the changes of cell morphology.[59,62,66]

***Adhesion***

Adhesion to a solid substratum is a particularly important step in cell migration.[14,65,67] It is mediated by integrins, which act as the primary receptors for extracellular matrix proteins and are consequently required for cell motility.[61,68] In addition, these molecules are also involved in signal transduction, and in regulating and stimulating migration.[68,69]

Adhesion and migration are inversely proportional; an optimal rate of migration is achieved with increasing adhesion, but mobility is reduced with further attachment.[61,68] Endothelial cells can adjust their adhesion intensity, with weakly adhesive cells moving faster than highly adhesive ones. After attachment to the extracellular matrix, the cell changes its morphology from an oval- or spindle-shape to an irregular, flattened one. These alterations in shape are governed by integrin signalling and depend on integrin contacts with the extracellular matrix in focal

complexes, forming initially at the ends of the filopodia.[61,67,68] Endothelial cells migrate fastest immediately after injury, then they enter a slower migration rate, which is maintained during the healing process.[66,70]

***Traction***

Contractile forces, transmitted through the integrin–cytoskeletal connections, allow the cell to pull the cytoplasm forward by generating traction to the substratum.[59] The force for movement is provided by myosin motor proteins, linked to contractile actin bundles along the cell. Interactions between myosin and actin fibres pull the cell body forwards. At the same time, the extracellular matrix-binding proteins on the trailing edge of the moving cell must release their connections.[67,68]

The degree of strength of the integrin coupling to the cytoskeleton is influenced by the rigidity of the substratum. With stronger couplings to a firm surface, force can be transmitted through the migrating cell more efficiently.[68] During locomotion, the traction forces generated at the sites of contact can be high enough to deform the extracellular matrix and to rearrange it significantly.[53 – 58] The direction of migration requires initial polarization of the cell and both physical and chemical stimuli influence it, as has been discussed above.[44,71 – 75]

Macrophages, proliferating fibroblasts and vascularized stroma, together with collagen matrix, fibrinogen, fibronectin and hyaluronic acid, constitute the acute granulation tissue that replaces the fibrin-based provisional matrix.[33,39] With collagen accumulation, the density of the blood vessels diminishes and the granulation tissue gradually matures to produce a scar.[51,64,76]

***Epithelialization***

Migration of epithelial cells starts from the wound edges within a few hours of wounding. A single layer of cells initially forms over the defect, accompanied by a marked increase in epithelial cell mitotic activity around the wound edges. Cells migrating across them attach to the provisional matrix below. When the advancing epithelial cells meet, migration stops and the basement membrane starts to form.[26,28,39]

## REMODELLING PHASE

As the final phase of wound healing, the remodelling phase is responsible for the development of new epithelium and final scar tissue formation. Synthesis of the extracellular matrix in the proliferative and remodelling phases is initiated contemporarily with granulation tissue development. This phase may last up to 1 or 2 years, or sometimes for an even more prolonged period of time.[33,35] The remodelling of an acute wound is tightly controlled by regulatory mechanisms with the aim of maintaining a delicate balance between degradation and synthesis, leading to normal healing. Along with intracellular matrix maturation, collagen bundles increase in diameter and hyaluronic acid and fibronectin are degraded.[29,39] The tensile strength of the wound increases progressively in parallel with collagen collection.[39,41] Collagen fibres may regain approximately 80% of the original strength compared with unwounded tissue. The acquired final strength depends on the localization of the repair and its duration, but the original strength of the tissue can never be regained.[3,41]

Synthesis and breakdown of collagen as well as extracellular matrix remodelling take place continuously and both tend to equilibrate to a steady state about 3 weeks after injury.[30,41] Matrix metalloproteinase

enzymes, produced by neutrophils, macrophages and fibroblasts in the wound, are responsible for the degradation of collagen. Their activity is tightly regulated and synchronized by inhibitory factors. Gradually, the activity of tissue inhibitors of metalloproteinases increases, culminating in a drop in activity of metalloproteinase enzymes, thereby promoting new matrix accumulation.[15,41,77]

Although the initial deposition of collagen bundles is highly disorganized, the new collagen matrix becomes more oriented and cross-linked over time. Its subsequent organization is achieved during the final stages of the remodelling phase, to a greater extent by the wound contraction that has already begun in the proliferative phase. The underlying connective tissue shrinks in size and brings the wound margins closer together, owing to fibroblast interactions with the extracellular matrix. The process is regulated by a number of factors, with PDGF, TGF-β and FGF being the most important.[41,42] As the wound heals, the density of fibroblasts and macrophages is further reduced by apoptosis.[40,76] With time, the growth of capillaries stops, blood flow to the area declines and metabolic activity at the wound site decreases.[39,41,78] The end result is a fully matured scar with a decreased number of cells and blood vessels and a high tensile strength.[78 – 80]

### HEALING BY PRIMARY AND SECONDARY INTENTION

Successful wound healing depends on the timely and optimal functioning of many diverse processes, cell types, molecular mediators and structural elements. Different cells dominate various phases of the repair, and cellular patterns vary according to the different types of injury and the extent of tissue damage.[3,81 – 85] During normal wound healing, closed incisions and open wounds with tissue defects progress through a series of co-ordinated molecular and cellular events, resulting either in regeneration or tissue repair. The least complicated example of wound repair is the healing of clean wounds without loss of tissue and uninfected surgical incisions approximated by sutures. This is referred to as healing by primary intention.[8,24,40] Death of a limited number of epithelial and connective tissue cells occurs because of small disruptions in basement membrane continuity. It is a fast process and contrasts markedly with the healing of an open wound with extensive loss of tissue.[8,10] Here, the reparative process is more complicated because large tissue losses have to be filled, which occurs during healing by secondary intention. In comparison to healing by first intention, this process takes longer and large amounts of granulation tissue are formed in order to fill the tissue defect.[3,9,33,52,84]

Numerous pathophysiological and metabolic factors can affect wound healing and result in a poor outcome.[26] They include local causes, such as oedema, ischaemia, tissue hypoxia, infection, necrosis and growth factor imbalance, as well as systemic causes including metabolic disease, nutritional status and general perfusion disturbances or pre-existing illness. These factors act by altering the wound repair environment, impeding healing and turning the acute wound into a chronic one. All processes, from wound closure time, inflammatory cell influx and fibroblast migration, to collagen and extracellular matrix deposition are delayed in this situation.[22,26,29 – 31,84]

## Management of wounds

The correct approach to treating wounds should effectively assist the healing process

and it can have an important impact on the final clinical outcome. Physiological, endocrine and nutritional support at a clinical level significantly influence repair and, without them, wound healing often fails completely.[6,24,29]

The first stage of wound management should be a thorough assessment of the wound and the patient. The process begins with a diagnosis of the wound's aetiology and continues with optimizing the patient's medical condition, particularly blood flow to the wound area.[26,85,86] An acute wound in a stable patient with normal blood flow should heal successfully if appropriate care is given. Guidelines of the medical procedures to use during wound management have been extensively described elsewhere.[6,10,20,23,25,28 – 30]

The wound will need to be debrided and dressed correctly.[29,33,46] Sufficient debridement, defined as the removal of non-viable, infected and hyperkeratotic tissue, forms the basis of non-delayed as well as delayed wound healing. Debridement is essential as it accelerates wound healing and different techniques exist.[26,86] In chronic wounds, the measures used to reverse medical abnormalities are complex and the aetiology of the wound is not easy to identify. Correct debridement helps to convert a chronic wound into an acute one, which can then progress through the normal stages of healing.[40,77,86,87]

The accumulation of devitalized tissue in the wound promotes bacterial colonization and impairs the body's ability to fight infection, thereby preventing complete repair of the wound.[3] The aim of debridement is to remove ischaemic and necrotic tissue, which presents potential for infection and contamination of the tissue by bacteria and foreign bodies. During the operation, necrotic and vital tissues are distinguished by a lack of capillary refill, colour and clear demarcation.[84 – 86] Dead muscle fibres do not contract on stimulation and are poorly perfused. Special tissues, such as tendons and fascia, are not removed despite not being vital because they promote the healing process in the wound; a surgeon does so only in case of severe contamination.[88,89]

The next important step is the lavage of micro-organisms, dead tissue and foreign bodies, which can further decrease tissue bacterial counts. Commonly, a bacitracin solution is used. In contrast, Patzakis[89] recommends irrigation with large quantities of saline. Low pressure irrigation only removes contamination on the surface. Although high pressure irrigation reduces bacterial colonization of the wound and the frequency of infections, a high pressure jet can damage fine tissues and push dirt particles deeper into the wound or even into the bone. High pressure irrigation also causes the soaking of wound margins with liquid, reducing the ability of the wound to resist infection. High pressure irrigation must be employed cautiously and is only recommended for very contaminated wounds.[88] Necrectomy and irrigation tend to complement each other. When it is not possible to irrigate all of the bacteria and dirt from the wound or the infection has spread, necrectomy removes contaminated and devitalized tissue, which would otherwise weaken the wound defence mechanisms.[88 – 90]

## Novel management options for wounds

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

of soft tissue damage and post-operative morbidity. Novel techniques of topical growth factor application and incisional priming with PDGF or IL-1 can optimize both the cellular and molecular environment, thus decreasing healing time by modifying inflammation and accelerating the proliferative phase.[91,92] Electrical field stimulation may optimize the remodelling phase by promoting more efficient fibroblast recruitment and collagen deposition,[93 – 96] prosthetic materials can favour tissue repair,[4,97] and gene therapy,[98] which is currently in pre-clinical development, may be able to provide a way for selective healing.

## Conclusion

Wounds occupy a remarkable place in everyday pathology. They are of diverse aetiology and different classification criteria exist. Any wounding damages the tissue and affects the local environment. The host's response to wounding involves various processes of tissue healing that are triggered by tissue injury, and encompasses four continuous phases including coagulation and haemostasis, inflammation, proliferation and wound remodelling with scar tissue deposition. Correct clinical management may positively influence the wound healing course and reduce potential complications.

## Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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Author's address for correspondence
**Dr Tomaz Velnar**
Department of Neurosurgery, University Medical Centre Maribor, Ljubljanska ulica 5, 2000 Maribor, Slovenia.
E-mail: t.velnar.s06@cranfield.ac.uk