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# WOUNDS

## The healing process

By SARAH COCKBILL, FCPP, FRPHARMS

This month's special feature focuses on wounds. The three articles consider the healing process, products to be included in a hospital dressings formulary, and the use of maggots in wound care

An ulcer on the heel: cellular, physiological, biochemical and molecular processes will be involved in repair

**O**ngoing research is opening up our understanding of wound healing and the pathogenesis of the mechanisms of chronic versus acute wound healing. Wound healing is a complex series of interrelated events which combine to return the damaged tissue to as near normal function as possible. It follows a specific sequence of phases which may overlap. The process of wound healing depends on the type of tissue which has been damaged and the nature of the tissue disruption. Deep open wounds in bone do not heal in the same way, or at the same rate, as superficial epithelial wounds, largely because bone "tissue" consists of up to 65 per cent inorganic, calcium-based matrix.

This article describes the wound healing process and outlines some of the cellular and acellular factors involved.

The objective of any wound management regimen is to heal the wound in the shortest

time possible and with minimum pain, discomfort and scarring to the patient. Success in fulfilling the objective will be assisted by an understanding of the healing process and a knowledge of the contributions that the existing range of wound management products can make to initiating and maintaining the optimal microenvironment for healing.

### STRUCTURE OF THE SKIN

**T**he skin is the largest organ of the body and its function is protective. It is composed of several layers: the outer epidermis and stratum corneum which protects against injury and contamination, the dermis which contains the capillary network providing nutrients and removing waste, the sensors for detecting pain and immediate environmental changes, and the subcutis from which the dermis and epidermis develop. The skin covers the other organs and, as well as the roles already mentioned, it plays a part in temperature regulation, detection of external pressure and external temperature changes, absorbs sunlight (thus aiding in its conversion to vitamin D) and acts as a

waterproof barrier.

The thickness of the epidermal layer varies from a thin membrane at internal flexures, eg, at the elbow, to thick, compacted layers at points which bear considerable pressure, eg, the palms and soles. The epidermal layer is crossed by hair follicles, sebaceous glands and sweat glands, which arise in the dermis.

The outer surface of the dermis (the papillary layer) is formed of ridges which project into the epidermis. The papillary layer contains blood vessels, lymphatics and nerve endings. The blood supply is distributed between outer vessels that nourish the epidermal cells and deeper vessels that lie just outside the lower subcutaneous layer of fat. The elasticity of skin, and its ability to retain and lose water rapidly, is the consequence of the presence of a network of collagen fibres. Beneath the dermis is the fat-containing subcutaneous layer, which is highly vascularised. The dermis insulates internal structures from excessive heat and reduces heat loss in cold climates. Due to its spongy texture and flexibility, it may also dissipate the effects of physical trauma.

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## CLASSIFICATION OF WOUNDS

Wounds can be defined as any process which leads to the disruption of the normal architecture of a tissue. They may be closed (eg, bruises, ruptures and sprains) or open (eg, abrasions, lacerations, avulsions, ballistic, hernias and excised or surgical wounds). Open wounds are by far the most common and are characterised by a break in the skin. Wounds need to be assessed continually at all stages of the healing process and an appropriate dressing regimen devised for the wound at that time, ie, they need to be "diagnosed" and have the appropriate material "prescribed" for that point of healing.

Wounds may be classified according to the number of skin layers affected. Damage limited to the epithelial tissue alone (epidermis) is regarded as a superficial wound which will heal rapidly by regeneration of epithelial cells. A partial thickness wound involves the deeper dermal layer and includes vessel damage. A full thickness wound affects the subcutaneous fat layer and beyond. Its healing will require the synthesis of new connective tissue and it takes longer to heal because it contracts, whereas partial thickness wounds do not.<sup>1,2</sup>

However, in the management of wounds and the use of wound management products, a further division of wounds into clean and non-infected is essential. Contaminated wounds should never be closed without thorough removal of all the damaged tissue (debridement), and even then it may be necessary to delay closure until the risks of infection have receded.

## THE PROCESS OF HEALING

Wound healing involves cellular, physiological, biochemical and molecular processes which result ultimately in connective tissue repair and the formation of a fibrous scar.<sup>3</sup>

All wounds heal using a combination of three mechanisms. Contraction is the major method by which wound healing occurs at an amputation site, such as the tip of a finger. Epithelialisation predominates in the healing of abrasions and connective tissue deposition occurs when lacerations are sutured closed.

**Healing by primary intention** If the two apposed surfaces of a clean, incised wound (which has not been subject to a significant degree of tissue loss) are held together, healing will take place from the internal layers outwards. The two surfaces may be held together through the use of sutures, wound management products or surface adhesives. The process of healing by first intention is initiated by the movement of epithelial cells from the two edges of the wound towards its

centre. The epithelial cells usually meet and commence interlocking within four to seven days of the incision. The reappearance of normal skin follows keratinisation and thinning of the epidermis. The pre-trauma strength of the tissue will never be completely regained.

**Healing by secondary intention** If there has been a significant loss of tissue in the formation of the wound, healing will begin by production of granulation tissue at the base of the wound. The process of healing by secondary intention always involves contraction of the wound. The degree of contraction is greatest during the first few days after the wound has been inflicted.

## THE STAGES OF HEALING

Wound healing starts immediately after damage has occurred, but the mechanism and speed of healing, and the eventual nature of the regenerated tissue, depend on the type of wound. The three main stages of healing are inflammation, repair (which may be further subdivided into proliferation and organisation) and maturation (regeneration).<sup>3,4</sup> In each of these stages, specific components play a part through several mediators.

Proliferation takes place through the actions of fibroblasts, epithelial and endothelial cells, and is largely dependent on growth factors and collagen deposition. Maturation is facilitated by collagen cross linking and collagen degradation, increasing scar strength as scar formation occurs.

In looking for the underlying engines of wound healing, attention is being focused on inflammatory cytokines, tumour necrosis factor and growth factors. Growth factors and cytokines are polypeptides transiently produced by cells and exert their hormone-like function on other cells via specific cell-surface receptors. Their activities overlap and the effect of most of them depends on the group and pattern of regulatory molecules to which the cell is exposed. Growth factors and cytokines are so named because of their stimulatory effect on cell proliferation. They display both stimulatory and inhibitory activities, even with the same cells, depending on the state of activation and differentiation of the cells and the presence of other stimulating factors. Unlike steroids, which penetrate the cell, growth factors precipitate their action after attachment to the cell membrane. They are either autocrine (acting on the cell that produced them), juxtacrine (acting on an adjacent cell), paracrine (acting on the local environment) or endocrine (acting on a distant cell). Very few effects in wound healing are due to growth factor activity in the endocrine category.

The mechanism of healing may be most usefully considered chronologically, although it must be remembered that the

process is continuous and well-defined stages do not occur in practice. The process of wound healing follows a specific sequence of phases which may overlap.

## THE INFLAMMATORY PHASE

Inflammation is a protective tissue response to an injury and this initial phase is the beginning of the healing process. It is characterised by pain, heat, redness, swelling and loss of function at the site of the wound. These classic signs of inflammation can be seen almost immediately after an injury and are characteristic of an impending wound infection. The purpose is to destroy, dilute or isolate the injurious agent and the injured tissues.

The drop in potential difference across the edges of the wound after injury acts as the stimulant for Hageman factor XII, which is responsible for activation of the healing cascade.<sup>5</sup> The effector systems within the cascade (the plasminogen cascade, the complement cascade, the kinin cascade and the clotting cascade) interlink to control infection and regenerate tissue. They release chemical mediators, such as complement C5a, fibrin degradation factors, platelet activity factors and vasoconstrictors, such as histamine and serotonin.<sup>4</sup>

Cessation of blood flow from the wound is achieved by vasoconstriction at the wound site and clot formation (haemostasis). Immediately following injury, platelets, endothelial cells, fibrin and fibronectin aggregate and release coagulation factors, cytokines and growth factors that are vital for haemostasis and initiation of the wound healing process. Cytokines are non-antibody proteins that are released from some cells and act as intracellular mediators, and include lymphokines and interleukins.

Inflammation continues through the action of neutrophils, macrophages and lymphocytes, mediated by growth factors and proteases. The platelets forming the clot are activated by exposure to collagen or microfibrils and to platelet-derived growth factor (PDGF) produced by the erythrocytes damaged during injury. The platelets adhere to the subendothelium exposed after injury, they then flatten and release a prostaglandin which encourages aggregation and, in combination with vasoconstrictors (histamine and serotonin from mast cells), reduce immediate blood loss prior to the initiation of the clotting cascade.<sup>6,7</sup> Activated platelets release several growth factors, ie, PDGF, platelet derived epidermal growth factor (PDEGF), epidermal growth factor (EGF), transforming growth factors  $\alpha$  and  $\beta$  (TGF- $\alpha$ , TGF- $\beta$ ), heparin-binding epidermal growth factor (HB-EGF) and insulin-like growth factor-1 (IGF-1), which stimulate cell growth and migration at the injured site from within their alpha granules. These growth factors stimulate the orderly migration of cells (neutrophils fol-

lowed by macrophages and then fibroblasts) into the wound site.<sup>8</sup> Activated platelets also stimulate the intrinsic coagulation system which converts fibrinogen to a fibrin mesh to produce a thrombus, stabilising the platelet plug.<sup>6,9,10</sup>

The clot maintains haemostasis and creates a provisional matrix for the migration of cells, ie, monocytes, fibroblasts and keratinocytes, to the wound with the consequent release of their cytokines and mediators. When blood flow is controlled, vasoconstriction is replaced by vasodilation. This allows for influx of a protein rich exudate containing antibodies and various complement fractions together with other substances essential to the healing process.<sup>8,11</sup>

This vasodilation is accompanied by increased vascular permeability, which results in plasma entering the site of injury via diapedesis (passage through blood vessel walls). Chemoattractants released by the platelets stimulate the rapid influx of neutrophils and monocytes from the circulation to the wound site.<sup>3</sup> Oedema is observed together with an increase in wound temperature and pain caused by the action of histamine, kinins and prostaglandins.<sup>12</sup> This permeability may last for up to one hour. The occlusion of the local wound lymphatic channels by fibrin prevents the spread of the inflammation. During inflammation, white blood cells (neutrophils) migrate to the wound area and, with macrophages, they ingest bacteria and cell debris by phagocytosis. The successful macrophage function normally indicates the end of the acute inflammatory reaction.

Neutrophils and monocytes have a common origin (a pluripotent bone marrow stem cell) and overlapping functions. Once the monocyte becomes phagocytic, it is referred to as a macrophage. Both macrophages and neutrophils are able to kill and digest bacteria and damaged tissue and thus help prevent infection by organisms that may be introduced into the host through the wound.

Neutrophils are short lived and die once they have phagocytosed bacteria and necrotic tissue, but they continue to aid healing as they release toxins which further

**False-colour scanning electron micrograph of a spreading macrophage. Macrophages are pivotal in bringing about the first stages of healing after which they control and direct the healing process** (CNRI/SCIENCE PHOTO LIBRARY)

stimulate the inflammatory response and contribute to the activation which produces macrophages from monocytes. Macrophages also serve as an important source of growth factors that regulate the wound healing response.<sup>6,7</sup> Macrophages are pivotal in bringing about the first stages of healing after which they then control and direct the process before finally stopping it when the repair is complete. The cells modulate the immune response by induction of lipoxygenase products through stimulation of the arachidonic acid cascade.<sup>9</sup> In addition to aiding debridement at the wound site, they are involved in the secretion and synthesis of the collagenases neutrophil elastase and matrix metalloproteinase eight (MMP 8) preparatory to laying down new extracellular matrix (connective tissue). They are another source of the growth factors PDGF and TGF- $\beta$  and regulate fibroblast migration and proliferation by production of the cytokine interleukin-1 $\beta$  (IL-1 $\beta$ ).<sup>13,14</sup>

The action of macrophages and polymorphs continues in all damaged tissues, irrespective of their degree of vascularisation, up to the fifth post-trauma day. Even if the wound is clean and the number of polymorphs is consequently reduced, the repair mechanisms will continue. However, if the

number of macrophages is reduced, healing will cease. This is thought to be a consequence of the directing role which macrophages undertake and of their involvement in the production of fibroblasts which synthesise collagen, the body's principal structural protein. The presence of collagen may be detected in fresh wounds from as early as the second day. Macrophages also stimulate new blood vessels from the surrounding tissue to grow into the wound. Their appearance heralds the next stage of healing.

#### THE REPAIR PHASE

The repair phase can be divided into two separate stages: proliferation and organisation.

**Proliferation** The inflammation phase overlaps the proliferation or repair phase. During the transition from inflammation to proliferation, the number of inflammatory cells

decreases while the number of fibroblasts within the wound site increases. Depending on the severity of the wound and its site, the proliferative phase may begin as early as the third day and last until the end of the third week. Fibroblasts are attached to the site and synthesise collagen, beginning on the fourth or fifth day of injury and continuing for two to four weeks. Specific chemoattractants, such as growth factors TGF- $\beta$  and PDGF, stimulate the influx of macrophages.<sup>13</sup> At the surface of the wound, epithelial cells are beginning to cover the tissue defect. The end of this phase is marked by the re-epithelialisation of the wound surface.<sup>15,16</sup>

The first stage of this process is to restore the vascular integrity (angiogenesis), which involves the migration, proliferation and organisation of endothelial cells under the influence of the following growth factors: acidic fibroblast growth factor (aFGF), tumour necrosis factor-beta (TNF- $\beta$ ), wound angiogenesis factor (WAF), vascular endothelial growth factor (VEGF) and EGF.

Capillaries are formed by endothelial budding with the production of granulation tissue and lysis of the previously produced fibrin network. This regeneration of capillaries and arterioles continues until equilibrium

of arterial and venous blood pressure is obtained within the microcirculation. Endothelial cells are organised so that developing tissues are ensured a supply of oxygen and nutrients. This is achieved as capillary loops infiltrate into the wound space.<sup>6,9,17</sup> Also during this phase, extracellular matrix (ECM) components are deposited to replace lost or damaged tissue. Connective tissue is synthesised and is known initially as granulation tissue. This happens around day four of healing.<sup>18,19</sup>

Granulation tissue is composed of macrophages, fibroblasts and neovasculature in a loose matrix which is subsequently covered by an epithelium. The macrophages produce cytokines which stimulate cells to activate fibroblasts, endothelial cells, and T and B cells.<sup>13</sup>

Fibroblasts are the major cell responsible for the production of tropocollagen, the precursor to collagen, elastin and the proteoglycans that make up the ECM in con-

nective tissue. Fibroblasts also secrete a range of growth factors that play a part in this phase of the wound healing process and these are insulin-like growth factor one (IGF-1), basic fibroblast growth factor (bFGF), TGF- $\beta$ , and keratinocyte growth factor (KGF). PDGF and bFGF stimulate connective tissue formation and directly enhance epithelialisation. EGF and TGF- $\beta$  increase the rate of epithelialisation while KGF stimulates keratinocyte proliferation.<sup>20,21</sup> Epithelial cells adjacent to the wound site are also an important source of these growth factors. Fibroblasts, in addition to macrophages, are a major source of matrix metalloproteinases (MMPs), which degrade the ECM at an appropriate point, and also of tissue inhibitors of MMPs (TIMPs). These are proteolytic enzymes responsible for the elimination of fibres which do not contribute to the structural strength of the wound.<sup>13,22-24</sup> As granulation is completed, the wound edges contract

thereby reducing the size of the defect. This is achieved by the transformation of fibroblasts to myofibroblasts, which contain contractile proteins. The degree of contraction varies with the depth of the wound. Vascularity is reduced and the inflammatory cells leave the healing site. Usually, a fine-line scar results.<sup>25-27</sup>

**Organisation** The organisation phase marks the period of regain of order within the wound, with the production of collagen reaching a peak from about day five to day seven. Fibroblasts act as the source of new collagen, with the greatest production occurring in a slightly acid environment. The importance of adequate nutrition in a patient at this stage is critical, as the presence of vitamin C (ascorbic acid) acts as an important stimulator of collagen manufacture. In its absence, newly formed blood vessels remain unsupported and subsequently break down, producing the

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characteristic appearance of purpura.

Endothelial cells produce buds which fuse and subsequently differentiate into blood vessels (ie, arterioles, capillaries, and venules). In an adequately nourished patient, these vessels are supported within the scaffolding of collagen fibres, producing granulation tissue. The amount of granulation directly correlates with the extent of inflammation which occurred in stage 1 of the healing process. This, in turn, depends on how effectively dead tissue, foreign bodies, and infection have been excluded from the wound site. The importance of a clean environment, a healthy immune response, and a well-nourished individual, cannot be over-stressed. The over-production of granulation tissue will result in an oversized and usually unsightly scar.

Epithelialisation varies between wound types. Wounds that contain little or no epithelial cells will be subject to a longer and more difficult process than superficial wounds. Sutured full thickness wounds will have new

epithelium within three days, although they will have little tensile strength. In partial thickness wounds, the epithelial cells from hair follicles and sebaceous glands migrate towards one another but, in full thickness wounds, where there is a lack of dermal appendages, epithelial cells must migrate from the edges of the wound. When they meet, contact inhibition halts their lateral proliferation but they continue to proliferate vertically to produce a multicellular layer which resurfaces the wound. Keratinocytes, epidermal cells which secrete keratin, migrate into the area within hours of the injury. They secrete membrane components, ie, fibronectin, collagen and laminin.<sup>28</sup>

The epithelialisation process is sensitive to environmental factors, such as pH, moisture and temperature, and deviation from the optimum values can have a detrimental effect upon healing. Once repair has taken place, the wound will still undergo a continued phase of regeneration

or maturation.<sup>13</sup>

#### THE MATURATION PHASE

The maturation/regeneration phase is the longest stage of wound healing and can last from three weeks to two years after the original injury. This phase overlaps with the repair phase and consists of a series of dynamic processes in which the ECM composition continually reflects a balance between synthesis and degradation of the components present in the wound. Fibroblasts produce tropocollagen molecules which combine to form collagen fibrils, filaments and fibres. There is an increase in the tensile strength of the wound which, in cavity wounds, is accompanied by contraction caused by modified fibroblasts called myofibroblasts.

Wound contraction is a natural healing process which allows open wounds to heal almost as rapidly as those which have been

sutured. It appears to be mediated by myofibroblasts which contain smooth muscle fibrils, although the process is not universally effective. As collagen is deposited the fibroblasts disappear. The wound will now be covered by epidermis and the maturation continues for up to two years; this varies between individuals and their age at the time of injury. Collagen type III, which is synthesised during granulation, is replaced by the stronger collagen type I and gives the tissue greater tensile strength. As this collagen develops, there is a decreased demand for oxygen and nutrients within the wound and therefore a reduction in the microvasculature. The new tissue which develops is known as scar tissue, and this will only ever reach between 70 per cent and 80 per cent of the original tissue strength.

#### EFFECTIVENESS OF HEALING

To produce effective wound healing, the body must supply a range of materials and nutrients to the site of damage. Factors which promote healing include an adequate blood supply and a healthy diet providing protein, vitamin A, vitamin C, zinc, and copper. Both systemic and local factors may challenge the successful continuation of the wound healing stages. Systemic factors include, in addition to nutritional status, concurrent therapy (such as corticosteroids, prostaglandin inhibition and oncolytic agents) and clinical conditions, such as anaemia and diabetes. These must be monitored and the objective must be the holistic management of the patient and not just the wound. The presence of wound infection is to be avoided at all costs.

Local factors which delay healing may be avoided by providing products which will produce the optimal microenvironment for healing.<sup>29</sup> This microenvironment should be moist at the wound interface but should remove excess exudate to avoid irritation and excoriation. The tissue temperature should be maintained and the injury protected from infective organisms, foreign particles and toxic compounds. In addition, when the dressing is changed there should be no secondary trauma due to adherence.

No single dressing will meet all the criteria required in all of the healing stages. Careful selection, based upon knowledge and experience, is necessary if rapid healing of the injury is to be achieved with minimum discomfort to the patient. In the future, bioactive products, such as growth factors which have a cellular activity and are used to potentiate one or more steps in the healing cascade, will be further developed for use in wound management for both humans and animals.

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