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The Physiology of Wound Healing

Introduction

Wound healing is a highly complex process. It is important that the nurse has an understanding of the physiological processes involved for several reasons:

- understanding the physiology of skin assists in understanding the healing process;
- an understanding of the physiology of wound healing makes it possible to recognise the abnormal;
- recognition of the stages of healing allows the selection of appropriate dressings;
- understanding of the requirements of the healing process means that appropriate nutrition can, as far as is possible, be given to the patient.

Definitions associated with wounds

Any damage leading to a break in the continuity of the skin can be called a wound. There are several causes of wounding:

- traumatic – mechanical, chemical, physical;
- intentional – surgery;
- ischaemia – e.g. arterial leg ulcer;
- pressure – e.g. pressure sore.

In both traumatic and intentional injury there is rupture of the blood vessels, which results in bleeding followed by clot formation. In wounds caused by ischaemia or pressure the blood supply is disrupted by local occlusion of the microcirculation. Tissue necrosis follows and results in ulcer formation, possibly with a necrotic eschar or scab.

Wounds in the skin, or deeper have been labelled in various ways. Some of them can be described as follows.

- (1) *Partial- and full-thickness wounds*
 - A partial-thickness wound is one where some of the dermis remains and there are shafts of hair follicles or sweat glands.
 - In a full-thickness wound all the dermis is destroyed and deeper layers may also be involved.
- (2) *Healing by first and second intention*
 - These definitions were first described by Hippocrates around 350 BC.
 - Healing by first intention is when there is no tissue loss and the skin edges are held in apposition to each other, such as a sutured wound.
 - Healing by second intention means a wound where there has been tissue loss and the skin edges are far apart, such as a leg ulcer.
- (3) *Open and closed wounds*
 - These are the same as healing by second and first intention respectively.

The structure of the skin

The skin is the largest and one of the most active organs of the body. It is composed of two layers: the epidermis and dermis with the epidermis forming the outer surface of the body and the dermis forming the deeper layer of the skin. The main structures of the skin can be found in the dermis. Figure 1.1 shows a cross-section of the skin.

Dermis

Dermis is composed of connective tissue, both collagen and elastic fibres, which is both elastic and resilient and provides support for the structures in the dermis. Blood vessels, lymph vessels, sensory nerve endings, sweat and sebaceous glands and hair follicles can be found within the dermis. The ducts of the glands and hair shafts pass through the epidermis to the skin surface. Sweat glands have their own ducts opening on the skin surface, but sebaceous glands open onto the hair follicles. The base or bulb of hair follicles is sited deep into the dermis. They are lined with epithelial cells and can play a role in the healing of partial-thickness wounds.

The surface of the dermis where it interlocks with the epidermis is irregular with projections of cells called papillae. The base of the dermis is less clearly defined as it blends into subcutaneous tissue, which contains both connective tissue and adipose tissue and helps to anchor the skin to muscle and bone.

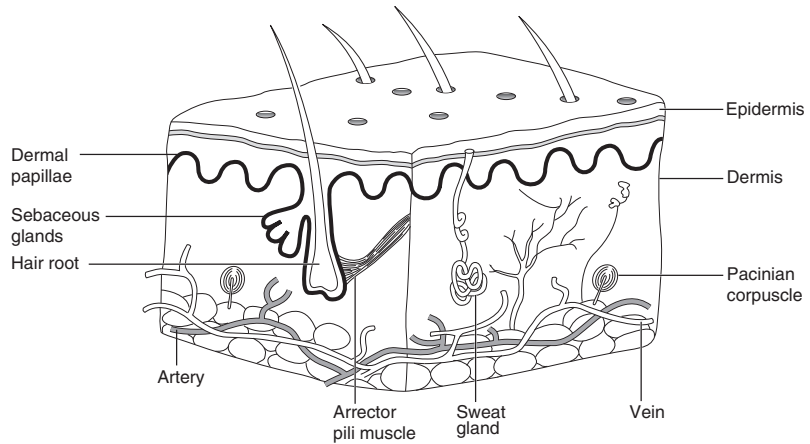


Figure 1.1 A cross-section of the skin

Epidermis

The epidermis comprises several layers of cells. The deepest layer is the *stratum basale* and it is constantly producing new cells by cell division. These cells are gradually pushed towards the skin surface taking about 7 weeks to reach the surface. The *stratum spinosum* contains bundles of keratin filaments, which hold the skin together. The top three layers of epidermis are the *stratum granulosum*, which produces the precursor to keratin, the *stratum lucidum* and the *stratum corneum*. As they move through the strata, the cells gradually flatten and the protoplasm becomes replaced with keratin. The cells in the *stratum corneum* are flat with no nucleus and are essentially dead cells. They are constantly worn away and replaced by new cells moving to the surface.

In addition the epidermis has cells called melanocytes, which contain melanin that gives skin its colour. A high concentration of melanin produces a dark skin colour. Ultraviolet light increases melanin production. This may occur naturally by sunlight resulting in a suntan or artificially such as a treatment in dermatology.

Wound healing

The wound healing process consists of a series of highly complex interdependent and overlapping stages. These stages have been given a variety of names. They are described here as:

- inflammation;
- reconstruction;

- epithelialisation;
- maturation.

The stages last for variable lengths of time. Any stage may be prolonged because of local factors such as ischaemia or lack of nutrients. The factors that can delay healing are discussed in more detail in Chapter 2.

Inflammation

The inflammatory response is a non-specific local reaction to tissue damage and/or bacterial invasion. It is an important part of the body’s defence mechanisms and is an essential stage of the healing process. The signs of inflammation were first described by Celsus, in the first century AD, as redness, heat, pain and swelling. The factors causing them are shown in Table 1.1.

When there is traumatic or intentional injury that causes damage to the blood vessels, the first response is to stop the bleeding. This is achieved by a combination of factors. First, by vasoconstriction that reduces the blood flow and second by the release of a plasma protein called von Willebrand factor from both endothelial cells and platelets, resulting in platelet aggregation and formation of a platelet plug. The third factor is the initiation of the clotting cascade and the development of a fibrin clot to reinforce the platelet plug.

Hageman factor (factor XII in the clotting cascade) triggers both the complement and kinin systems. The complement system consists of plasma proteins, which are inactive precursors. When activated, there is a cascade effect that leads to the release of histamine and serotonin from the mast cells and results in vasodilation and increased capillary permeability. The complement system also assists in attracting neutrophils to the wound. The complement molecule, C3b, acts as an opsonin, that is, it assists in binding neutrophils to bacteria. Five of the proteins activated during the cascade process form the membrane attack complex, which has the ability to directly destroy bacteria.

Table 1.1 The signs of inflammation

Signs and symptoms	Physiological rationale
Redness	Vasodilation results in large amount of blood in the area
Heat	Large amount of warm blood and heat energy produced by metabolic reactions
Swelling	Vasodilation and leakage of fluid into the wound area
Pain	May be caused by damage to nerve ends, activation of the kinin system, pressure of fluid in the tissues or the presence of enzymes, such as prostaglandins, which cause chemical irritation

The effect of the complement system is enhanced by the kinin system, which, through a series of steps, activates kininogen to bradykinin. Kinins attract neutrophils to the wound, enhance phagocytosis and stimulate the sensory nerve endings. The apparent delay in feeling pain after injury is explained by the short time lag taken for the kinin system to be activated.

As the capillaries dilate and become more permeable, there is a flow of fluid into the injured tissues. This fluid becomes the 'inflammatory exudate' and contains plasma proteins, antibodies, erythrocytes, leucocytes and platelets. As well as being involved in clot formation, platelets also release fibronectin and growth factors called platelet-derived growth factor (PDGF) and transforming growth factor alpha and beta (TGF α and TGF β). Their role is to promote cell migration and growth at the wound site.

Growth factors are a subclass of cytokines, proteins that are used for cellular communication (Greenhalgh, 1996). The particular role of growth factors is to stimulate cell proliferation. There are a number of growth factors involved in the healing process, and they are listed in Table 1.2. Some growth factors have been isolated and used as a treatment for chronic wounds. This will be discussed in more detail in Chapter 4.

The first leucocyte to arrive at the wound is the neutrophil. Fibronectin attracts neutrophils to the wound site, a process known as chemotaxis. Neutrophils squeeze through the capillary walls into the tissues by diapedesis, again this ability is enhanced by fibronectin. Within about an hour

Table 1.2 Growth factors involved in the healing process

Growth factor	Abbreviation	Action
Platelet-derived growth factor	PDGF	Chemotactic for neutrophils, fibroblasts and, possibly, monocytes. Encourages proliferation of fibroblasts
Transforming growth factor alpha	TGF α	Stimulates angiogenesis
Transforming growth factor beta	TGF β	Chemotactic for monocytes (macrophages). Encourages angiogenesis. Regulates inflammation
Fibroblast growth factor	FGF	Stimulates fibroblast proliferation and angiogenesis
Epidermal growth factor	EGF	Stimulates the proliferation and migration of epithelial cells
Insulin-like growth factors	IGF-I, IGF-II	Promote protein synthesis and fibroblast proliferation. Work in combination with other growth factors
Vascular endothelial growth factor	VEGF	Critical for angiogenesis and the formation and growth of blood vessels

of the inflammatory response being initiated, neutrophils can be found at the wound site. They arrive in large numbers, their role being to phagocytose bacteria by engulfing and destroying them. Neutrophils decay after phagocytosis as they are unable to regenerate the enzymes required for this process. As the numbers of bacteria decline, so too, do the numbers of neutrophils.

Transforming growth factor beta attracts monocytes to the wound where they differentiate into macrophages. Fibronectin binds onto the surface receptors on the cells promoting diapedesis and phagocytosis. Oxygen is vital to this process and macrophages can be inactivated and their ability to undertake phagocytosis reduced if the partial oxygen pressure falls below 30 mmHg (Cherry *et al.*, 2000). Macrophages are larger than neutrophils and so are able to phagocytose larger particles, such as necrotic debris, as well as bacteria. The lifespan of the neutrophil can be a few hours or a few days. When they die they are also phagocytosed by the macrophages.

T lymphocytes also migrate into the wound, although in smaller numbers than macrophages (Martin & Muir, 1990). They influence macrophage phagocytic activity by the production of several macrophage-regulating factors. They also produce colony-stimulating factors that encourage the macrophage to produce a range of enzymes and cytokines. One such substance is prostaglandins, which maintains vasodilation and capillary permeability. It can be produced on demand to prolong the inflammatory response if required. A study by Martin and Muir (1990) found that both macrophages and lymphocytes are present in wounds from day 1, with macrophages peaking between days 3 and 6 and lymphocytes between 8 and 14 days.

Mast cells play a supporting role in the healing process (Ng, 2010). They produce a range of growth factors (PDGF and TGF β 1), inflammatory mediators interleukin 1 (IL-1), tumour necrosis factor alpha (TNF α) and proteases (chymase and tryptase). Chymase and tryptase assist in the breakdown of the extra-cellular matrix in anticipation of the phase of reconstruction.

Inflammation lasts about 4–5 days. It requires both energy and nutritional resources. In large wounds the requirements may be considerable. If this stage is prolonged by irritation to the wound, such as infection, foreign body or damage caused by the dressing, it can be debilitating to the patient as well as delaying healing.

Reconstruction

The reconstruction phase is characterised by the development of granulation tissue. It consists of a loose extracellular matrix of fibrin, fibronectin, collagen and hyaluronic acid and other glycosaminoglycans. Macrophages and fibroblasts and the newly formed blood vessels can be found within this matrix. Macrophages play a major role in this phase of healing. They

produce PDGF and fibroblast growth factor (FGF), which are both chemotactic to fibroblasts, attracting them to the wound and stimulating them to divide and later to produce collagen fibres. Fibronectin has been shown to play a role in enhancing fibroblast activity (Kwon *et al.*, 2007). Collagen has been seen in a new wound as early as the second day. Collagen fibres are made up of chains of amino acids in a triple helix formation. There are a number of different types of collagen characterised by different formations of amino acids. Type III is present in the healing wound in greater proportions than would normally be found in skin. Over time, this proportion reduces in favour of higher levels of type I collagen.

Fibroblasts are key cells in this phase of healing (Harding *et al.*, 2002). As well as being responsible for the production of collagen, they also produce the extracellular matrix, which is seen visually as granulation tissue. Tryptase from the mast cells also supports deposition of collagen into the extracellular matrix (Abe *et al.*, 2002). As new extracellular matrix is synthesised, the existing matrix is degraded by enzyme systems such as matrix metalloproteinases (MMPs). There are a number of MMPs, in particular MMP-1, MMP-2 and MMP-9, involved in the healing process, although their role is imperfectly understood at present.

The activity of fibroblasts depends on the local oxygen supply. If the tissues are poorly vascularised the wound will not heal well. The wound surface has a relatively low oxygen tension, encouraging the macrophages to produce TGF β and FGF, which instigates the process of angiogenesis, the growth of new blood vessels. Undamaged capillaries beneath the wound sprout buds, which grow towards the surface and loop over and back to the capillary. The loops form a network within the wound supplying oxygen and nutrients. Vascular endothelial growth factor (VEGF) produced within the extracellular matrix is responsible for controlling blood vessel formation and growth (Schultz & Wysocki, 2009).

Some fibroblasts have a further role, they are involved in the process of contraction. The exact process is not clearly understood and there are currently two theories postulated: cell contraction and cell traction. The theory of cell contraction is based on specialised fibroblasts known as myofibroblasts and was proposed by Gabbiani *et al.* in 1973. Myofibroblasts have a contractile apparatus, similar to that in smooth muscle cells. In *in vitro* models, they have been shown to cause contraction of the wound. Tomasek *et al.* (1989) found a higher level of contractile forces when a high level of myofibroblasts was present. The concept of cell traction was put forward by Stopak and Harris (1982), who demonstrated that fibroblasts could contract collagen gels by a physical pull, resulting in a rearrangement of the extracellular matrix. Dalton and Ehrlich (2008) reviewed the use of fibroblast-populated collagen lattices to study the process of contraction. As well as myofibroblasts and the concept of tractional forces they describe the mechanism of cell elongation, which also can cause contraction provided there is a high density of fibroblasts. In his review of the role of the mast cell,

Ng (2010) noted that mast cells also seem to be essential for wound contraction. It must be noted that all these studies were undertaken *in vitro* and there is no certainty that they could be repeated *in vivo*.

Whatever the actual process, contraction may start at around the fifth or sixth day. It considerably reduces the surface area of open wounds. Irvin (1987) suggests that contraction could be responsible for as much as 40–80% of the closure. It is certainly of considerable importance in large cavity wounds. However, in shallower wounds with a large surface area such as burns, contraction may lead to contractures. Myofibroblasts disappear after healing is completed.

In wounds healing by first intention, little can be seen of this stage of healing. But in those healing by second intention, the granulation tissue can be seen as it gradually fills the wound cavity. They are followed by capillary buds growing towards the areas of low oxygen tension in the wound.

As the wound fills with new tissue and a capillary network is formed, the numbers of macrophages and fibroblasts gradually reduce. This stage may have started before the inflammation stage is completed and prolonged inflammation can result in excessive granulation with hypertrophic scarring. The length of time needed for reconstruction depends on the type and size of wound, but may be about 24 days for wounds healing by first intention.

Epithelialisation

This phase describes the phase whereby the wound is covered with epithelial cells. Macrophages release epidermal growth factor (EGF), which stimulates both the proliferation and migration of epithelial cells. Keratinocytes at the wound margins and around hair follicle remnants synthesise fibronectin, which forms a temporary matrix along which the cells migrate. The cells move over the wound surface in a leapfrog fashion, the first cell remaining on the wound surface and forming a new basement membrane. When cells meet, either in the centre of the wound, forming islets of cells, or at the margin, they stop. This is known as contact inhibition. Epithelial cells only move over viable tissue and require a moist environment (Winter, 1962). In sutured wounds, epithelial cells also migrate along the suture tracks. They are either pulled out with the sutures, or gradually disappear.

Once the cells stop moving on the wound surface, they start to reconstitute the basement membrane, which is essential in order for the epidermis to 'fix' to the dermis. Until the basement membrane is fully reconstituted it is easy for epithelial cells to be sheared off the wound surface by mechanical forces (Cherry *et al.*, 2000).

Epithelialisation commences as early as the second day in closed wounds. However, in open wounds it is necessary for the wound cavity to be filled with granulation tissue before it can commence. There is a very variable time span for this stage.

Maturation

During maturation the wound becomes less vascularised as there is a reduction in the need to bring cells to the wound site. The collagen fibres are reorganised so that, instead of being laid down in a random fashion, they lie at right angles to the wound margins. During this process, collagen is constantly degraded and new collagen synthesised. The highest level of activity in this process occurs between days 14 and 21 (Cherry *et al.*, 2000). The scar tissue present is gradually remodelled and becomes comparable with normal tissue after a long period of time. The scar gradually flattens to a thin white line. This may take up to a year in closed wounds and very much longer in open wounds.

Tensile strength gradually increases. This is a way of describing the ability of the wound to resist rupture or dehiscence. Forester *et al.* (1969) found that at 10 days an apparently well-healed surgical incision has little strength. During maturation it increases so that by 3 months the tensile strength is 50% that of normal tissue. Further work by Forester *et al.* (1970) compared surgical incisions where the skin edges were held together by tape with those where sutures were used. The findings showed that, when tape was used, the wounds regained 90% strength of normal tissue, whereas sutured wounds only regained 70% strength.

Impaired wound healing

Although the majority of wounds heal without problem, impaired healing may sometimes occur. Some of the different types of impaired healing are described here. Their management will be discussed elsewhere.

Hypertrophic scars

Hypertrophic scars are more common after traumatic injury, especially large burns. They occur shortly after the injury or surgery and remain limited to the area of the injury. They are raised scars with increases in pigmentation, vascularity and pliability (Oliviera *et al.*, 2009). However, they will generally flatten out with time; about 1–2 years.

Van der Veer *et al.* (2009) suggest that an overabundant production of extracellular matrix results in hypertrophic scars that can easily be recognised by their stiffness and rough texture and their colour mismatch. They reviewed all possible activity at the cellular and molecular level to identify any potential causes of this type of scarring and concluded that a number of factors were involved including an increase in the levels of fibronectin, histamine, TGF β , PDGF, MMPs, IL-4 and IL-13. The impact of this is increased proliferation of fibroblasts and extracellular matrix deposition and reduced collagen breakdown. However, it must be noted that it is still

uncertain whether these changes are the cause or effect of scar formation (Van der Veer *et al.*, 2009).

Oliviera *et al.* (2009) compared the levels of types I and III collagen in hypertrophic and normal scars of male children with burns of over 40% of total body surface area. Scars on the thigh following deep burns were studied at 12, 18 and 24 months. Wound biopsies were taken and the collagen levels measured. They found that there was a higher level of accumulation of type III collagen in the deep dermal layer of the skin in the hypertrophic scars when compared with normal scars. There was no difference in type I collagen.

Keloids

Keloids are similar to hypertrophic scars in that they are also the result of an excessive fibrous response. Keloids take some time to form and may occur years after the initial injury. They can range in size from small papules to large pendulous growths (Munro, 1995). Keloids more commonly occur in individuals aged between 10 and 30 years (Cosman *et al.*, 1961) and in those with a darker skin (Placik & Lewis, 1992). Unfortunately, unlike hypertrophic scars, keloids do not gradually flatten out.

Within keloids there are increased levels of collagen and glycosaminoglycan deposition within the extracellular matrix with the collagen presenting as thickened whorls of collagen bundles laid down in a very haphazard manner (Robles *et al.*, 2007). The precise pathogenesis is still unknown, although overexpression of a number of growth factors such as PDGF, TGF α and TGF β has been identified. In normal healing, there is a negative feedback system to reduce fibroblast proliferation as healing completes. It is proposed that this negative feedback mechanism is deficient in keloidal fibroblasts, allowing scar formation to persist (Robles *et al.*, 2007). Ogawa (2008) has proposed an alternative theory that keloids arise because of a mechanoreceptor or a mechanosensor disorder and that mechanical force or stretching of the skin may be a major causative factor.

Contractures

Wound contraction is part of the normal healing process, but occasionally contraction will continue after re-epithelialisation has occurred resulting in scar contraction (Tredget *et al.*, 1997). Contractures can occur in any wound, but they are more likely if there is delayed healing or in burns (Lee & Clark, 2003). There can be considerable restriction of movement if contractures occur over a joint.

Hildebrand *et al.* (2008) used an animal model to study cellular changes in the presence of contractures and found raised levels of myofibroblasts, TGF β , MMP-1 and MMP-13 as well as reduced levels tissue inhibitor of

metalloproteinases (TIMPs) and changes in collagen structure. The significance of these changes has yet to be ascertained.

Acute to chronic wounds

Chronic wounds may be called chronic because their underlying aetiology makes healing a very long process. A good example is the venous leg ulcer. However, some chronic wounds may have originally been acute wounds that have failed to heal over a long period of time, perhaps years. The original factor delaying healing may have been related to infection or local irritation, perhaps caused by a suture. Once these problems have been resolved the wound still fails to heal causing considerable misery to the patient.

The differences between acute and chronic wounds are still imperfectly understood. However, work by Phillips *et al.* (1998) did shed some light on the problem. They used cultured fibroblasts from human neonatal foreskin as a plated laboratory model and treated them with either chronic wound fluid (CWF) or bovine serum albumen (the control). They found that CWF inhibited the growth of the fibroblasts quite dramatically. The researchers concluded that this study gave some indication of how the microenvironment of a chronic wound has a negative effect on the healing wound. As result of this work, other research groups have looked at wound exudate in more detail.

Trengrove *et al.* (1999) used wound fluid from venous leg ulcers at both non-healing and healing stages to measure MMP levels. They found elevated levels of MMPs at the non-healing stage, which decreased significantly as the ulcers started to heal ($p = 0.01$). The levels of MMPs in the healing ulcers were similar to those in acute wounds, thus suggesting that failure to heal may be linked to excessive matrix degradation. Ladwig *et al.* (2002) collected wound fluid from 56 pressure ulcers and found lower levels of MMP-9 in those ulcers that went on to heal well compared with those that healed poorly.

Trengrove *et al.* (2000) undertook further studies of wound exudate from non-healing and healing leg ulcers. They found significantly higher concentrations of a number of pro-inflammatory cytokines or growth factors in the non-healing ulcers. They consider that wound healing is delayed in chronic wounds because of an impairment of inflammatory mediators rather than any deficit of growth factors.

Subramaniam *et al.* (2008) compared wound fluid from non-healing venous leg ulcers, mastectomy wounds and donor sites to determine MMP levels, TIMPs levels and fibroblast activity. They found a significantly higher level of MMP-1 and MMP-3 production by dermal fibroblasts in the chronic venous leg ulcer fluid compared with the acute wound fluid. There was variation in TIMP-1 levels as the level was very low in both the chronic leg ulcer fluid and the acute graft sites and high in the acute

mastectomy fluid. The authors concluded that this could be the result of several variables including the types of wounds and the methods used to collect the wound fluid. Further research is required to obtain greater understanding.

Premature ageing of fibroblasts may also be a problem. Mendez *et al.* (1998) investigated the characteristics of fibroblasts cultured from chronic venous ulcers and found signs of accelerated ageing or senescence in these cells. Senescent fibroblasts have reduced mobility, are less able to replicate, have abnormal protein production and do not respond well to growth factors. A small study of seven patients by Stanley and Osler (2001) compared the senescence rates in fibroblasts taken from chronic venous ulcers with fibroblasts taken from punch biopsies taken from the proximal thigh of the same patient. They found a significantly higher senescence rate in the fibroblasts from the leg ulcers ($p = 0.0001$). Wall *et al.* (2008) found that fibroblasts exposed to chronic wound fluid had a decreased ability to withstand oxidative stress resulting in premature senescence. Telgenhoff and Shroot (2005) suggest this is related to the chronic inflammation found in chronic wounds.

Conclusion

This chapter has described 'normal' physiology. However, not all wounds heal without complication or delay and some of the differences between acute and chronic wound healing have been discussed. But many factors can affect the healing process and they will be considered in more detail in Chapter 2.

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