The physiology of wound healing

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Abstract

Wound healing is a complex biological process which results in the restoration of tissue integrity. Physiologically, it can be broken down into four distinct phases of haemostasis, inflammation, proliferation and tissue remodelling. This article describes the cellular basis of wound healing and the extracellular signalling processes which control them. The function of platelets, neutrophils, macrophages and fibroblasts are considered in detail. The concept of healing by primary and secondary intention is discussed. Many factors are known to adversely affect healing including malnutrition, hypoxia, immunosuppression, chronic disease and surgery. It is essential that surgeons understand the key physiological processes involved in healing in order to minimize patient morbidity from delayed healing.

Keywords Haemostasis; inflammation; proliferation; tissue remodelling; wound healing

Introduction

Disruption of the integrity of skin, mucosal surfaces or organ tissue results in the formation of a wound. Wounds can occur as part of a disease process or have an accidental or intentional aetiology.[1] At the time of insult, multiple cellular and extracellular pathways are activated, in a tightly regulated and coordinated fashion, with the aim of restoring tissue integrity. Classically, this process of wound healing is divided into four distinct phases: haemostasis, inflammation, proliferation and tissue remodelling. Given the intricate nature of the healing cascade, it is remarkable how often it occurs without complication. Many factors can interfere with this process, resulting in delayed wound healing, increased patient morbidity and mortality and poor cosmetic outcome. The health economic effects of chronic wounds and the psychological sequelae for the patients are often understated as they are difficult to quantify completely. It has been estimated, however, that the annual expenditure on wound-related problems in the USA alone exceeds one billion dollars.[2] The aim of this article is to provide surgeons with a basic overview of the physiology of wound healing, discuss the cellular mechanisms involved in each of the four phases and highlight the clinical factors, which may contribute to wound complications.

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Acute and chronic wounds

Regardless of the aetiology of the wound, the repair processes are similar. Acute wounds are typically due to some form of trauma that could be blunt or penetrating (surgical incisions, gunshots, animal bites, etc.). A wound results in tissue damage that stimulates a coordinated physiological response to provide haemostasis and initiate the processes of inflammation, proliferation and remodelling.[3] Acute wounds, including surgical incisions, usually pass through these phases relatively quickly. Wounds that demonstrate delayed healing 12 weeks after the initial insult are termed chronic wounds, often as a result of prolonged pathological inflammation. Surgical incisions are usually clean and cause minimal tissue loss and disruption. Surgical wounds are a controlled form of trauma that can be categorized on the basis of degree of contamination(i.e. clean, clean contaminated, contaminated and dirty) to predict the risk of wound infection following surgery. These wounds can be closed immediately with sutures and tend to heal rapidly. This is termed as closure by primary intention. When the wound is contaminated and left open to prevent infection and wound closure is performed after a few days, it is termed as delayed primary healing. When the tissue loss has been more extensive, the edges cannot be approximated, or the wound must be left open due to sepsis, the reparative process is prolonged, as the defect must fill with extensive granulation tissue. This process is termed as closure by secondary intention. Huge defects can heal in this manner, but the end cosmetic result is often inferior to those closed primarily (Figure 1).

Haemostasis

At the time of surgical incision, vascular injury occurs on a macro- or microvascular scale. The immediate response of the body is to prevent exsanguination and promote haemostasis. Damaged arterial vessels rapidly constrict through the contraction of smooth muscle

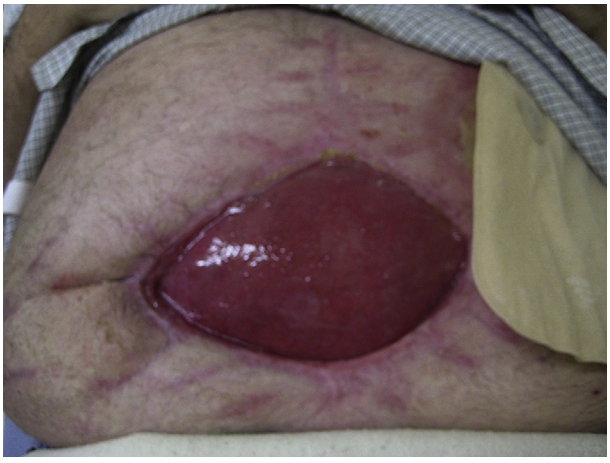

Figure 1 An open laparostomy wound that has closed through secondary intention. Note the large area of granulation tissue.

in the circular layer of the vessel wall, mediated by increasing cytoplasmic calcium levels.[4] Vessels up to a diameter of 5 mm can be completely closed through contraction, although this can only occur if the injury is in a transverse plane. Within a few minutes, the reduced blood flow mediated by arteriole constriction leads to tissue hypoxia and acidosis. This promotes the production of nitric oxide, adenosine and other vasoactive metabolites to cause a reflex vasodilatation and relaxation of the arterial vessels. Simultaneously, histamine release from mast cells also acts to increase vasodilatation and increase vascular permeability, facilitating the entry of inflammatory cells into the extracellular space around the wound. This explains the characteristic warm, red, swollen appearance of early wounds.

Further blood loss at this stage is also prevented through the formation of a clot which is achieved through three key mechanisms:

1. Intrinsic pathway of the clotting cascade (contact activation pathway) — Endothelial damage as a result of tissue injury exposes the sub-endothelial tissues to blood which results in the activation of factor XII (Hageman factor). This initiates the proteolytic cleavage cascade which results in the activation of factor X which converts prothrombin to thrombin resulting in the conversion of fibrinogen to fibrin and the formation of a fibrin plug.
2. Extrinsic pathway of the clotting cascade (tissue factor pathway) — Endothelial damage results in exposure of tissue factor (which is present in most cells) to circulating blood. This results in activation of factor VII and the rest of the extrinsic pathway of the clotting cascade which eventually results in thrombin activation.
3. Platelet activation — Following activation by thrombin, thromboxane or ADP, platelets undergo a change in morphology and secrete the contents of their alpha and dense granules.[5] Activated platelets adhere and clump at sites of exposed collagen to form a platelet plug and temporarily arrest bleeding. This plug is strengthened by fibrin and von Willebrand factor as well as the actin and myosin filaments within the platelets.

Platelets are unnucleated fragments of bone marrow megakaryocytes. They have a crucial role in wound healing process. Not only are they essential for clot formation, they also produce multiple growth factors and cytokines which continue to regulate the healing cascade. Over 300 signalling molecules have been isolated from activated platelets, which influence and modulate the function of other platelets, leukocytes and endothelial cells.[6] The main actions of platelet-derived molecules are listed in Table 1. In addition to these factors, in response to the injured cell membranes caused by the wounding stimulus, arachidonic acid is broken down into a number of potent signalling molecules such as the prostaglandins, leukotrienes and thromboxanes which have roles in stimulating the inflammatory response.

Growth factors involved in wound healing

Factor	Released from	Action
TGF-α	Macrophages	Formation of granulation tissue
	Platelets	Stimulates proliferation of epithelial cell and fibroblasts
TGF-β	Platelets	Chemotaxis
	Neutrophils	Transdifferentiation of
	Macrophages	fibroblasts to myofibroblasts
	Fibroblasts	Collagen matrix construction
		Stimulates angiogenesis
		Wound contraction
		Release of other growth factors
		MMP stimulation
PDGF	Platelets	Chemotaxis
	Fibroblasts	Fibroblast proliferation
	Endothelial cells	Collagen deposition
	Macrophages	
VEGF	Platelets	Stimulate angiogenesis
	Neutrophils	Neovascularization
	Keratinocytes	
Serotonin	Platelets	Vasoconstriction
		Platelet aggregation
		Chemotaxis
		Increase vascular permeability
TNF-α	Platelets	Chemotaxis
		Nitric oxide release
		Activation of other growth factors
PGE_2	Keratinocytes,	Vasodilation
	Macrophages,	Platelet disaggregation
	Endothelial cells	Increased vascular permeability
		Pain
		Fever
Thromboxane A_2	Platelets	Platelet aggregation
		Vasoconstriction
Leukotrienes	Platelets	Increased vascular permeability
	Leukocytes	Chemotaxis
		Leukocyte adhesion
		Chemotaxis (neutrophils)
Interleukin-1	Platelets	Chemotaxis
	Endothelial cells	
	Lymphocytes	
Lipoxins	Platelets	Dampen inflammatory response
	Leukocytes	Inhibit chemotaxis (neutrophils)
Interferon-γ	Fibroblasts	Macrophage maturation
	Lymphocytes	Nitric oxide release

TGF: Transforming growth factor; PDGF: Platelet derived growth factor; VEGF: Vascular endothelial growth factor; TNF: Tumour necrosis factor; PGE_2: Prostaglandin E_2; FGF: Fibroblast growth factor; EGF: Epidermal growth factor; MMP: matrix metaloproteinases.

Table 1

Inflammation

The key aim of this stage of wound healing is to prevent infection. Regardless of the aetiology of the wound, the mechanical barrier which was the frontline against invading microorganisms is no longer intact. Neutrophils, the 'first responders', are highly motile cells which infiltrate the wound within an hour of the insult and migrate in sustained levels for the first 48hours. This is mediated through various chemical signalling mechanisms, including the complement cascade, interleukin activation and TGF-B signalling, which leads to

neutrophils passing down a chemical gradient towards the wound, a process termed chemotaxis.[3] Neutrophils have three main mechanisms for destroying debris and bacteria. Firstly they can directly ingest and destroy foreign particles, a process termed phagocytosis. Secondly, neutrophils can degranulate and release a variety of toxic substances (lactoferrin, proteases, neutrophil elastase and cathepsin) which will destroy bacteria as well as dead host tissue. Recent evidence has shown that neutrophils can also produce chromatin and protease 'traps' which capture and kill bacteria in the extracellular space. Oxygen free radicals are produced as a by-product of neutrophil activity, which are known to have bactericidal properties but can also combine with chlorine to sterilize the wound. When the neutrophils have completed their task, they either undergo apoptosis, are sloughed from the wound surface or are phagocytosed by macrophages.

Macrophages are much larger phagocytic cells that reach peak concentration in a wound at 48–72 hours after injury. They are attracted to the wound by the chemical messengers released from platelets and damaged cells and are able to survive in the more acidic wound environment present at this stage.[1] Macrophages harbour a large reservoir of growth factors, such as TGF-β and EGF, which are important in regulating the inflammatory response, stimulating angiogenesis and enhancing the formation of granulation tissue. Lymphocytes appear in the wound after 72 hours and are thought to be important in regulating wound healing, through the production of an extracellular matrix scaffold and collagen remodelling. Experimental studies have shown that inhibition of T-lymphocytes results in decreased wound strength and impaired collagen deposition.[7] A summary of the cells involved in inflammation is shown in Table 2.

The inflammatory phase of wound healing will persist as long as there is a need for it, ensuring that all excessive bacteria and debris from the wound is cleared. Protracted inflammation can lead, however, to extensive tissue damage, delayed proliferation and result in the formation of a chronic wound. Multiple factors, including lipoxins and the products of arachidonic acid metabolism, are thought to have anti-inflammatory properties, which dampen the immune response and allow the next phase of wound healing to arise.[8]

Cells involved in wound healing

Cell type	Time of action	Function
Platelets	Seconds	Thrombus formation Activation of coagulation cascade Release inflammatory mediators (PDGF, TGF-β, FGF, EGF, histamine, serotonin, bradykinin, prostaglandins, thromboxane.
Neutrophils	Peak at 24 hours	Phagocytosis of bacteria Wound debridement Release of proteolytic enzymes Generation of oxygen free radicals Increase vascular permeability
Keratinocytes	8 hours	Release of inflammatory mediators Stimulate neighbouring keratinocytes to migrate Neovascularization
Lymphocytes	72–120 hours	Regulates proliferative phase of wound healing although exact mechanisms are unclear Collagen deposition
Fibroblasts	120 hours	Synthesis of granulation tissue Collagen synthesis Produce components of extracellular matrix Release of proteases Release of inflammatory mediators

Table 2

Proliferation

Once the injuring stimulus has ceased, haemostasis has been achieved, the inflammatory response is balanced and the wound is debris free, the proliferative stage of the healing cascade can begin to repair the defect. This complex process incorporates angiogenesis, the formation of granulation tissue, collagen deposition, epithelialization and wound retraction which occur simultaneously.

Angiogenesis

Angiogenesis is triggered from the moment the haemostatic plug has formed as platelets release TGF-β, PDGF and FGF. In response to hypoxia, VEGF is released which, in combination with the other cytokines, induce endothelial cells to trigger neovascularization and the repair of damaged blood vessels. Mixed metalloproteinase (MMP) are a family of enzymes that are activated by invading neutrophils in hypoxic tissue. They promote angiogenesis through liberation of VEGF and remodelling of the extracellular matrix (ECM).[9] Initially the centre of the wound is relatively avascular, as it relies solely on diffusion from the undamaged capillaries at the wound edge. As the process of angiogenesis proceeds, a rich vascular network of capillaries is formed throughout the wound from offshoots of healthy vessels. Initially the capillaries are fragile and permeable which contributes further to tissue oedema and the appearance of healing granulation tissue.

Fibroblast migration

Following the wound insult, fibroblasts are stimulated to proliferate by growth factors released from the haemostatic clot and then migrate to the wound (predominantly by TGF-β and PDGF). By the third day, the wound becomes rich in fibroblasts which lay down extracellular matrix proteins (hyaluronan, fibronectins and proteoglycans) and subsequently produce collagen and fibronectin. The resulting pink, vascular, fibrous tissue which replaces the clot at the site of a wound is termed granulation tissue. This is composed of a different range of collagens (a higher proportion of type 3 collagen) to that seen in unwounded tissue. Once sufficient matrix has been laid down, fibroblasts change to a myofibroblast phenotype and develop pseudopodia. This enables them to connect to the surrounding proteins fibronectin and collagen and assist in wound contraction. Myofibroblasts also promote angiogenesis through mediation MMP activity.[10] Collagens synthesized by fibroblasts are the key component in providing strength to tissues. In wounds closed by primary intention, collagen deposition

is maximal by day 5 and this can often be palpated beneath the skin as a 'wound ridge'. When a wound ridge is not palpable, this is an indication that the wound is at risk of dehiscence. Overproduction of collagen can lead to the development of a hypertrophic scar. Hypertrophic scars remain raised and erythematous but remain within the confines of the original wound. Risks for their development include wound infections and those where there is excessive tension.

Epithelialization

Epithelial cells migrate from the edges of the wound very soon after the initial insult until a complete sheet of cells covers the wound and attaches to the matrix below. An embryological process, termed epithelial-mesenchymal transition (EMT), allows epithelial cells to gain motility and travel across the wound surface.[11] In wounds that are primarily closed, this phase can be completed within 24 hours. Changes in cytokine concentration result in epithelial cells switching from a motile phenotype to a proliferative one in order to repopulate epithelial cell levels and complete wound repair.[12] In wounds that heal by secondary intention, the area lacking epithelial cells can be large and the wound must contract significantly before epithelialization can be completed. In some cases this may never occur and skin grafting can be used to cover the defect.

Wound retraction

Wounds begin to contract about seven days after injury, mediated mainly by myofibroblasts. Interactions between actin and myosin pull the cell bodies closer together decreasing the area of tissue needing to heal. Contraction can occur at a rate of 0.75 mm per day leading to shortened scars. This is influenced by numerous factors including wound shape, with linear wounds contracting fastest and circular wounds the slowest. Disorders of this phase of healing can lead to deformity and the formation of contractures.[13]

Remodelling

The final stage of wound healing can take up to 2 years and results in the development of normal epithelium and maturation of the scar tissue. This phase involves a balance between synthesis and degradation, as the collagen and other proteins deposited in the wound become increasingly well organized. Eventually they will regain a structure similar to that seen in unwounded tissue (replacing type 1 collagen with type 3 collagen). Despite this, wounds never achieve the same level of tissue strength, on average reaching 50% of the original tensile strength by 3 months and only 80% long term. As the scar matures, the level of vascularity decreases and the scar changes from red to pink to grey with time.

Important factors in wound healing

Nutrition

It has long been recognized that nutritional status can influence wound healing. In the fifteenth century, the Portuguese explorer Vasco de Gama noted that sailors with scurvy had multiple, non-healing skin lesions. It was not until 1747 that James Lind, a Scottish surgeon, demonstrated that citrus fruits could successfully treat scurvy and enhance wound repair. Malnutrition adversely affects healing by prolonging inflammation, inhibiting fibroblast function and reducing angiogenesis and collagen deposition. There are many essential nutrients which are important for wound healing, including vitamin A (involved in epidermal growth), carbohydrates (for collagen synthesis) and omega-3 fatty acids (modulate arachidonic acid pathway). In recent years, extensive research in the field of clinical nutrition has shown clear benefit for the use of nutritional support techniques to enhance wound healing. This topic has been the subject of a number of recent review articles.[14]

Hypoxia

All wounds are hypoxic to some extent as their local vascular supply is disrupted. While a degree of hypoxia is required to facilitate re-epithelialization, sufficient oxygen is an essential requirement for wounds to heal. It is clear in surgical practice that elderly patients and those with peripheral vascular disease have poor healing and in contrast hyperbaric oxygen improves wound healing. Although hypoxia is one of the chemoattractants for neutrophils and macrophages, oxygen is needed to allow phagocytosis and for their optimal function. A randomized controlled trial demonstrated that supplemental oxygen given during the perioperative period reduced the risk of wound infections.[15] Oxygen is also essential for collagen deposition as it acts as a substrate in the hydroxylation of proline and lysine residues.

Smoking

Smoking impairs wound healing by its effects on chemotaxis, migratory function and oxidative bactericidal mechanisms in the inflammatory phase. In addition, it also reduces fibroblast migration and proliferation. Furthermore, smoking affect immune function, downregulates collagen synthesis and deposition.[16]

Infection

Antibiotic prophylaxis prior to making a surgical incision was proven to reduce risk of wound infections firstly in guinea pigs in 1958 and subsequently in humans in 1960. Delayed primary closure, or closing by tertiary intention, should be considered when suturing heavily contaminated wounds as this has been shown to decrease wound infection rates.

Immunosuppression

Patients with HIV, cancer and malnutrition all have a degree of immunosuppression which can lead to delayed wound healing. In addition, any drugs which impair the inflammatory response can impede the healing cascade. Oral steroids, such as prednisolone, have been shown to decrease cytokine concentrations during wound repair, leading to reduced collagen deposition.

Chemotherapy and radiotherapy may also have a negative effect on the process of wound healing. Chemotherapy medications affect vascular endothelial growth factor (VEGF) that is an important regulator in the angiogenesis phase of wound healing.[17]

Radiation injury to overlying skin causes tissue ischemia and may lead to skin ulcers. Surgical incisions over irradiated are more likely to develop wound complication and these wounds heal very slowly.[18]

Chronic disease

Any chronic disease which affects the cardio-respiratory system may adversely affect the supply of oxygen and other nutrients

required for wound healing. Patients with diabetes have significantly impaired wound healing as they are relatively immunocompromised and higher blood glucose levels affect leukocyte function. MMP expression and function is altered in diabetes, which contributes to poor wound healing to many other vascular diabetic complications.[19] Diabetes also causes long-term microvascular damage which affects both tissue oxygen levels and the supply of nutrients.

Wound management

A healthy wound environment is a prerequisite for successful wound healing. There are more than 250 different types of wound dressing which act to protect the wound, allow it to remain moist and absorb excessive exudates to aid the healing process. These are discussed in greater depth later in this issue.

Age

Elderly patients have a thinner epidermal layer and have slower inflammatory, migratory and proliferation responses. They are also more likely to have chronic disease, which combine to make these patients have slower wound healing and so be at higher risk of wound complications such as dehiscence.

Genetics

Keloid scars occur when there is an overgrowth of scar tissue which extends beyond the wound edges. They can be painful and itchy and have a high recurrence rate but can respond to steroids, cryotherapy or radiation therapy. They most commonly occur on shoulders, arms or upper chest and are rare below the waist. There is a strong genetic component to keloid development, being significantly more commonly in patients of Black, Hispanic or Asian race. Incisional herniae have also been shown to have a genetic component, which is thought to be due to defects in collagen deposition, with higher levels of type 3 collagen associated with hernia development.[20]

Surgical technique

Surgical technique is clearly vital in optimizing wound healing. Careful tissue handling, strict aseptic techniques, avoidance of tension across the wound and choice of suture will all contribute to minimizing wound complications. Intraoperative hypothermia should be avoided and supplemental oxygen should be give postoperatively to reduce infective complications.

Conclusions

Wound healing is a complex process requiring the coordinated actions of multiple cell types in response to a variety of differing cytokines and micro-environmental conditions. It is essential that surgeons understand the key physiological processes involved and the important factors which can influence these such that successful wound healing can be maximized to reduce morbidity and mortality from disturbed wound healing. ◆

REFERENCES

1 Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. *J Int Med Res* 2009 Sep-Oct; **37:** 1528–42.

2 Ueno C, Hunt TK, Hopf HW. Using physiology to improve surgical wound outcomes. *Plast Reconstr Surg* 2006 Jun; **117**(7 Suppl)**:** 59S–71.

3 Broughton G, Janis JE, Attinger CE. The basic science of wound healing. *Plast Reconstr Surg* 2006 Jun; **117**(7 Suppl)**:** 12S–34.

4 Khalil RA, van Breemen C. Mechanisms of calcium mobilization and homeostasis in vascular smooth muscle and their relevance to hypertension. In: Laragh JH, Brenner BM, eds. Hypertension: pathophysiology, diagnosis and management. New York: Raven Press, 1995; 523–40.

5 Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev* 2007 Mar; **21:** 99–111.

6 Smyth SS, McEver RP, Weyrich AS, et al. 2009 platelet colloquium participants. Platelet functions beyond hemostasis. *J Thromb Haemost* 2009 Nov; **7:** 1759–66.

7 Peterson JM, Barbul A, Breslin RJ, Wasserkrug HL, Efron G. Significance of T-lymphocytes in wound healing. *Surgery* 1987 Aug; **102:** 300–5.

8 Nathan C. Points of control in inflammation. *Nature* 2002 Dec 19-26; **420:** 846–52.

9 Siefert SA, Sarkar R. Matrix metalloproteinases in vascular physiology and disease. *Vascular* 2012 Aug; **20:** 210–6. 1708-5381; 1708–5381.

10 Mayrand D, Laforce-Lavoie A, Larochelle S, et al. Angiogenic properties of myofibroblasts isolated from normal human skin wounds. *Angiogenesis* 2012; **15:** 199–212.

11 Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell* 2008 Jun; **14:** 818–29.

12 De Donatis A, Ranaldi F, Cirri P. Reciprocal control of cell proliferation and migration. *Cell Commun Signal* 2010 Sep 7; **8:** 20.

13 Hinz B. Masters and servants of the force: the role of matrix adhesions in myofibroblast force perception and transmission. *Eur J Cell Biol* 2006; **85:** 175–81.

14 Campos A, Groth A, Branco A. Assessment and nutritional aspects of wound healing. *Curr Opin Clin Nutr Metab Care* 2008; **11:** 281–8.

15 Kurz A, Sessler DI, Lenhardt R. Perioperative normothermia to reduce the incidence of surgical-wound infection and shorten hospitalization. Study of Wound Infection and Temperature Group. *N Engl J Med* 1996 May 9; **334:** 1209–15.

16 Sørensen LT. Wound healing and infection in surgery: the pathophysiological impact of smoking, smoking cessation, and nicotine replacement therapy: a systematic review. *Ann Surg* 2012; **255:** 1069.

17 Erinjeri JP, Fong AJ, Kemeny NE, et al. Timing of administration of bevacizumab chemotherapy affects wound healing after chest wall port placement. *Cancer* 2011; **117:** 1296.

18 Mendelsohn FA, Divino CM, Reis ED, Kerstein MD. Wound care after radiation therapy. *Adv Skin Wound Care* 2002; **15:** 216.

19 Tsioufis C, Bafakis I, Kasiakogias A, Stefanadis C. The role of matrix metalloproteinases in diabetes mellitus. *Curr Top Med Chem* 2012; **12:** 1159–65. 1568-0266;1873–4294.

20 Henriksen NA, Yadete DH, Sorensen LT, Agren MS, Jorgensen LN. Connective tissue alteration in abdominal wall hernia. *Br J Surg* 2011 Feb; **98:** 210–9.

