The physiology of wound healing

Daniel Harper
Alistair Young
Clare-Ellen McNaught

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.

Daniel Harper ***FRCA*** *is a Specialist Registrar in Anaesthetics at Scarborough Hospital, Scarborough, UK. Conflicts of interest: none declared.*

Alistair Young ***MRCS PhD*** *is a Specialist Registrar in Surgery at Scarborough Hospital, Scarborough, UK. Conflicts of interest: none declared.*

Clare-Ellen McNaught ***FRCS MD*** *is a Consultant Colorectal Surgeon at Scarborough Hospital, Scarborough, UK. Conflicts of interest: none declared.*

Acute and chronic wounds

Regardless of the aetiology of the wound, the repair processes are similar. A wound results in tissue damage which 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. These wounds can be closed immediately with sutures and tend to heal rapidly. This is termed closure by primary intention. 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 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 macrovascular 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 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:

- 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.
- 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

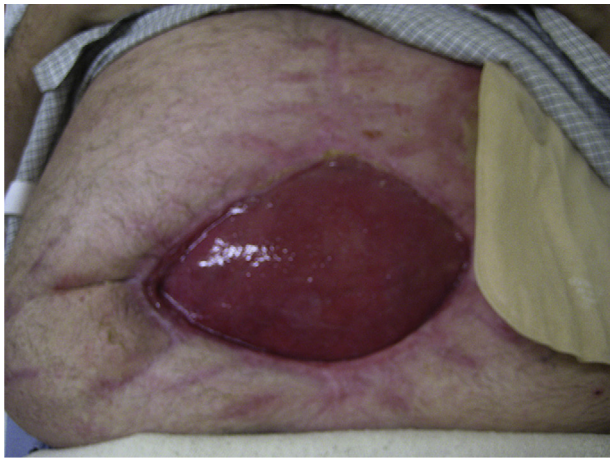

Figure 1 An open laparostomy wound that has closed through secondary intention. Note the large area of granulation tissue.

of the extrinsic pathway of the clotting cascade which eventually results in thrombin activation.

- 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 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.

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 micro-organisms 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 48 hours. This is mediated through various chemical signalling mechanisms, including the complement cascade, interleukin activation and transforming growth factor beta (TGF-β) 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 bacteriocidal properties but can also combine with chlorine to sterilize the wound. When the neutropils 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 which 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]

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, platelet-derived growth factor (PDGF) and fibroblast growth factor. In response to hypoxia, vascular endothelial growth factor (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