Mechanisms of Wound Healing

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Introduction

A wound is generally defined as a pathological state in which tissues are separated from each other and/or destroyed. This event is associated with a loss of substance and impairment of function. Wounds can occur in all tissues of the body but most often affect the skin. The term "wound" is most frequently used to describe damage of the body's outer covering, whereas the term injury tends to be used more for damage to internal organs. Wounds heal by the same biochemical mechanisms in all tissues. Wounds to the skin and their healing are described below as examples.

Learning Objectives

After completing this module, you will be better able to:

- Describe the difference between regeneration and repair.
- Discuss the body's vascular response to an acute injury.
- Explain the process of blood coagulation in provisional wound closure.
- Identify the function of the inflammatory process in the nonspecific immune defense process.
- Explain the role of fibroblasts and myofibroblasts in wound contraction.
Forms of Wound Healing

The body naturally attempts to close a wound and restore the functions of the damaged tissue as quickly as possible. All tissues of the body are capable of wound healing with the sole exception of the teeth. The original state can be restored by two different mechanisms: regeneration or repair.

Figure 1. Possible Forms of Wound Healing

Move through the following animation describing repair and regeneration by clicking on Next.

The tissue is destroyed by an injury and substance is lost.
Regeneration Versus Repair

Regeneration
Regeneration refers to the tissue-specific replacement of a lost part of the body or organ. The animal kingdom contains many examples of this process, e.g., the regeneration of a complete earthworm from the anterior segments of its body or the complete replacement of a severed extremity in the salamander.

Among mammals, and especially in man, complete regeneration is only possible in epithelial tissue (e.g., epidermis, mucous membranes of the gastrointestinal tract and female genitals) and, to a limited degree, in parenchymatous organs such as the liver.

Repair
Thus, in man, tissue defects are remedied mainly by repair. In this process, lost or damaged tissue is replaced by nonspecific elements of connective and supportive tissue which form a scar. Defects in the tissue, e.g., bones, cartilage and tendons, are regenerated with the typical structure of the tissue.

Wound healing can, therefore, be defined as the closure of a defect by scar-forming tissue associated with epithelial regeneration, i.e., epithelialization. Its aim is to restore the form and functions of the damaged tissue.
Physiology of Wound Healing

How does the body respond to a bleeding skin wound? Two main problems first have to be solved: the invasion of infectious organisms must be halted and bleeding stopped. Once this has been accomplished, foreign bodies and tissue debris have to be broken down and new tissue produced. In simplified terms, the processes involved can be divided into four phases:

- vascular response
- blood coagulation
- inflammation
- formation of new tissue.

These phases overlap and are to some extent interdependent.
**Vascular Response**

A fresh wound usually bleeds rather profusely if cutaneous and deeper lying blood vessels are damaged. The bleeding has the effect of cleansing the wound as the blood washes foreign bodies and organisms away.

To prevent further blood loss, the affected vessels narrow within a few minutes of the injury occurring. This *vasoconstriction* lasts for only a few minutes or long enough for the leaks to be sealed by blood clots. The ends of the affected vessels also turn inwards, as shown in Figure 2.

**Figure 2. The Vascular Response**

*Move through the following animation describing events following damage to a blood vessel by clicking on Next.*

![After injury of a cutaneous vessel, blood flows into the wound gap.](image)
Vascular Response (Continued)

Vasoconstriction is followed by vasodilation (widening of the vessels), reaching a maximum after about 10 minutes. Vasodilation increases the blood circulation in the wound area. This causes an increased production of heat and an associated rise in the temperature of the skin around the wound. In this phase, the permeability of the capillary walls to components of the blood is increased. As a result, blood constituents such as erythrocytes, leucocytes and platelets enter the wound. Furthermore, the increased capillary pressure associated with vasodilation allows increased amounts of blood plasma to escape into the interstitium.

Finally, vascular stasis (stoppage) occurs and persists for several hours. The oxygen deficiency in the tissue causes the CO₂ pressure to rise and, consequently, the pH falls into the acidic range. This acidosis causes a charge reversal of the chemical components in the connective tissue. As a result, the collagen components depolymerize and lose their capacity to bind water. The accumulation of fluid, especially the plasma, within the wound causes swelling of the collagen fibers. The outcome of all these processes, i.e., the intensified blood flow, the increased leakage of blood from the capillaries and the accumulation of fluid in the tissue, is wound edema.

Obviously, these vascular responses do not take place in an uncoordinated manner. They are controlled by a number of substances. Platelets adhering to the damaged vessels release thromboxane A₂ which causes vasodilation. The tissue hormones, histamine and serotonin, that are released by the mast cells present in the connective tissue and especially in the vicinity of small vessels, stimulate the dilation of the capillaries. They also cause the endothelial cells in the vessels to move apart, thereby increasing their permeability to blood components.

Mast cells are in turn stimulated to release histamine and serotonin by other substances in the wound area released from various cells and the blood plasma. These mediators include the complement factors in the blood serum. Complement factors are made up of about 209 proteins which are involved in the defense against foreign bodies. Other mediators are the prostaglandins (e.g., PGF2a) which are hormone-like substances particularly important for the inflammation process.
**Blood Coagulation**

Damage to the skin results in the wound rapidly filling with blood. A process called coagulation (clotting) then occurs which involves a large number of chemical reactions resulting in a scab which provisionally closes the defect. The scab protects the wound, reducing the risk of infection and dehydration. Figure 3 illustrates how a fresh wound fills with blood which coagulates and temporarily closes the defect.

**Figure 3. Provisional Wound Closure by Scab Formation**

Underlying this process of blood coagulation are complicated biochemical reactions involving various factors:

- the blood vessel walls
- the platelets
- damaged connective tissue cells
- the coagulation system.
Blood Coagulation (Continued)

Coagulation begins both in the opened vessels and in the wound gap. It starts when cells are damaged and release certain substances (mediators) that activate the biochemical reactions necessary for blood clot formation.

When a vessel wall is damaged, blood platelets stimulated by mediators such as Von Willebrand factor immediately adhere to the exposed collagen of the vessel wall. In this activated state, the platelets first change their form from flat disks to spherical structures with long extended processes known as pseudopods. These activated platelets also release a number of substances that induce further reactions (the coagulation "cascade"). At first, even more platelets from the blood adhere to those that are already adhering to form a clot. This process, known as platelet aggregation, is shown in Figure 4. Platelet aggregation is initiated by adenosine diphosphate (ADP), thromboxane A\textsubscript{2} and platelet activating factor (PAF) released from the activated platelets.

Figure 4. Aggregated Platelets Form a Thrombus in a Vessel

The clustered platelets partially coalesce with each other and release the platelet factors that initiate the actual clotting process. During the clotting process a fine line network of fibrin forms around the platelet plug and finally fills the entire wound gap. The purpose of this fibrin network is to "catch" erythrocytes and other solid components of the blood, retain them and thereby form a clot that provisionally seals the wound against the external environment and stops the bleeding. Its surface rapidly dries in the air and a dense protective scab forms.
Blood Coagulation (Continued)

Flowing blood understandably contains no solid fibrin, only its water-soluble precursor, fibrinogen. Fibrinogen is only converted to fibrin at the wound surface by the enzyme thrombin. This is also present in the blood as an inactive precursor called prothrombin. Prothrombin and fibrinogen are among the coagulation factors whose biochemical interaction ultimately leads to the formation of the blood clot. The clotting factors are distinguished by Roman numerals; fibrinogen (I) and prothrombin (II) are part of what is referred to as the coagulation cascade, a complex chain reaction which is set in motion by injury.

Coagulation is initiated first by the platelet factors released from the adhering platelets and then by substances liberated from damaged tissue cells. These thromboplastins activate the conversion of prothrombin into thrombin with the involvement of various other coagulation factors and calcium ions. Thrombin is now able to convert the water-soluble fibrinogen present in the blood into fibrin monomers. These polymerize spontaneously to fibrin chains which are finally interlinked by coagulation factor XIII to form the stable fibrin mesh.

Fibrinogen, prothrombin and the other coagulation factors are formed in the liver. The synthesis of prothrombin and three other factors is vitamin K dependent. This is why vitamin K deficiency leads to disturbances of blood coagulation.

On the other hand, this dependence can also be utilized therapeutically. For example, for thrombosis or infarction prophylaxis, vitamin K antagonists (e.g., coumadin derivatives) are administered to prolong the coagulation time, leading to a fall in prothrombin levels and delayed clot formation.

Inhibitors of the coagulation factors present in the blood ensure that coagulation is restricted to the wound area in the event of injury. They inactivate the thrombin molecules entering the circulation. The most important of these inhibitors is antithrombin III.
Inflammation

The substances released from the cell debris resulting from tissue destruction are responsible for causing the characteristic inflammatory reactions. The vascular changes previously described also contribute to this reaction. They create the preconditions for the body's inflammation:

- redness (rubor)
- heat (calor)
- swelling (tumor)
- pain (dolor)
- functional disturbance (functio laesa).

**Inflammation is not the same as infection.** Redness and heat are consequences of the increased blood flow in the wound area, causing an influx of immune defense cells into the site of injury.

**Figure 5. Wound Edema**

This photograph of edema after the skin has sustained a bleeding wound displays the classic signs of the inflammatory process, i.e., redness (rubor), swelling (tumor) and functional disturbance (functio laesa) with associated heat (calor) and pain (dolor).
Inflammation (Continued)

Wound Edema
The swelling or wound edema results from collections of fluid in the soft tissue. These accumulations of fluid in the tissue exert increased pressure on the small nerves and nerve endings causing the wound to hurt. The pain, in turn, causes the inflamed part of the body to assume a protective posture. This and the disturbed physiological processes account for the functional disturbance of the injured organ.

The inflammatory reaction is induced independently of invasion by foreign organisms. Therefore, "sterile" inflammations can also develop in closed injuries, e.g., bruises in which the skin remains intact. Open skin wounds are usually contaminated. Even in surgical wounds, the invasion by millions of microorganisms cannot be prevented even under the strictest sterile conditions. In such cases infection can develop initiating both the cellular and humoral immune responses.

Non-Specific Immune Defense
In the inflammatory phase, the number of white blood cells increases, manifested as leucocytosis in the blood count. The defense cells migrate from the blood vessels that have become permeable into the wound area. They are attracted by complement factors, which are part of the nonspecific defense system. These are proteins present in the blood serum in an inactive form which are activated by invading pathogens. The presence of pathogens initiates what is known as the complement cascade, i.e., complement factors become active and, in turn, activate other components of the pathway, finally resulting in a complement "attack complex" which either destroys the microorganisms directly or prepares them for destruction by other defense cells (opsonization). The activated complement factors also produce attractants (chemotaxins) which draw wandering phagocytes into the injured area.

First to appear at the site of inflammation is a type of granulocyte called neutrophils. These have the capacity to phagocyte (engulf and digest) foreign bodies. They also release enzymes that breakdown degenerating connective tissue (collagenases, elastase). Soon afterwards, monocytes arrive on the scene, attracted by chemotratcants released by aggregating platelets, i.e., platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β). Both of these growth factors stimulate the growth of new tissue. Monocytes are also capable of phagocytosis and once they have ingested foreign bodies they transform into macrophages. Macrophages produce a large number of substances which act as mediators of other wound healing processes and attract to the wound area further phagocytic cells. For example:

- growth factors TGF-a, TGF-β (transforming growth factor alpha and beta), TNF-a (tumor necrosis factor alpha) and FGF (fibroblast growth factor) that promote vascular and connective tissue neogenesis.
- prostaglandins that sustain the inflammatory process and influence vascular dilation.
- complement factors C1-C5 responsible for non-specific defense.
- interleukin-1 which induces fever and attracts further neutrophil granulocytes.

Macrophages also release enzymes which can destroy tissues. Necrotic tissue and dead phagocytic cells filled with foreign bodies form "pus" which surrounds the wound.
Inflammation (Continued)

Specific Immune Defense
In addition to the nonspecific immune defense provided by the complement system and phagocytes, the body is also capable of mounting a specific, targeted defense against a particular pathogen. It does this by producing substances called antibodies. Antibodies are synthesized by B-lymphocytes in response to specific foreign bodies (antigens) and released into the blood.

Antibodies are proteins which belong to a chemical class known as globulins and are, therefore, referred to as immunoglobulins. They have the ability to bind specific antigens to form antigen-antibody complexes, thereby rendering them harmless. Immunoglobulins are divided into subgroups depending on their structure and size: IgG, IgA, IgM, IgD and IgE. These different subgroups have different functions. For example, IgM antibodies are specifically directed against viruses while IgE antibodies are involved in allergic reactions.

Immunoglobulins circulate freely in the serum component of the blood. If they meet their corresponding antigen, for example in a wound, they bind it and either inactivate it directly or mark it for destruction by phagocytes.

B-lymphocytes continually produce antigen specific antibodies over a period of years and often for a lifetime, resulting in the production of "memory cells". If the same microorganism enters the body again at some later stage, the memory cells divide with great rapidity. The newly formed B-lymphocytes transform into plasma cells and synthesize large amounts of this specific antibody to eliminate the invaders rapidly and effectively. In this way, immunity against the respective pathogen is created. This mechanism is utilized in inoculation. By non-hazardous contact with attenuated or killed pathogens, the B-lymphocytes are stimulated to produce corresponding antibodies which protect the inoculated person from an outbreak of the infectious disease on renewed contact.

Other immunocompetent cells involved in specific defense are the T-lymphocytes. These differ from the B-lymphocytes in that they do not produce antibodies but, as "killer cells", identify and selectively destroy foreign cells. This is done through direct cell contact or by release of cell toxins, complement factors or hydrolytic enzymes. As with the B-lymphocytes, certain killer cells specialize in specific pathogens which they are able to identify by their surface properties. Here, too, there are memory cells that provide large numbers of specialized T-lymphocytes for defense in the event of antigen contact.

T-lymphocytes, differentiated into helper cells, assist the maturation of the B-lymphocytes into plasma cells and promote their antibody production. A further T-lymphocyte subclass, the suppressor cells, regulate the action of the T-helper cells and B-lymphocytes and thereby prevent an excessive immune response.

To summarize, the inflammatory phase of wound healing is characterized by an influx of granulocytes, macrophages and lymphocytes that absorb and enzymatically degrade foreign matter and tissue detritus and, thereby, clean the wound. An inflammatory reaction is also induced irrespective of whether an infection is present, e.g., in closed injuries such as bruises and contusions.
Whereas catabolic processes predominate in inflammation, the next phase of wound healing is characterized mainly by repair, i.e., anabolic reactions.
Formation of New Tissue

The cleansed wound, provisionally sealed by a scab (dry crust of serous exudate with leukocytes), can now be covered by new tissue. Various preconditions for this have already been created.

- Mediators and growth factors released from different cells in the wound area (e.g., TNF-α and TGF-α) activate vascularization (the formation of new blood vessels).
- Blood platelets and macrophages release growth factors (TGF-β and PDGF) that stimulate the production and influx of fibroblasts.
- The fibrin network acts as a guide structure for the cells moving into the wound area.

Vascularization

The precondition for favorable wound healing is the presence of sufficient blood circulation. This is why new blood vessels begin to grow into the wound only three days after an injury. The vascular buds are formed by existing intact vessels. Stimulated by growth factors and other substances released in the wound, the endothelial cells in the venules begin to produce enzymes that break down the basal membrane in the area of the stimulus. Endothelial cells then migrate through the resulting gap in the direction of the wound. They divide and form tubular structures that connect with other buds. During the maturation process a new basal membrane develops from the extracellular matrix components. The newly formed vascular loops then connect with intact vessels and differentiate accordingly into arterioles or venules. Superfluous vessels are broken down again.

Figure 6. Stages of New Vessel Formation

Move through the following animation describing the stages of new vessel formation by clicking on Next.
Formation of New Tissue (Continued)

Parallel to vascularization and also proceeding from the wound margins, new tissue is formed. Fibroblasts, attracted chemotactically, migrate along the fibrin network and divide at a rapid rate. They produce the connective tissue ground substance consisting of proteoglycans (protein network with carbohydrate side chains) as well as the water-soluble collagen fibers essential for tissue stability.

Collagen is a fibrous protein synthesized in several stages. Its precursors are assembled from amino acids in the endoplasmic reticulum of the fibroblast.

These protocollagen chains are twisted together in threes in helical formation and then transferred to the Golgi complex. Here the chains are re-arranged slightly and interlinked more closely. Golgi vesicles finally transport the molecules to the cell membrane where they are released as soluble tropocollagen into the intercellular space. Here the tropocollagen molecules accumulate to form protofibrils which then polymerize into microfibrils.

Several microfibrils unite to form a collagen fibril, several of which, in turn, arrange themselves into bundles. In healthy tissue, the collagen fibers are aligned in certain patterns following the main contours of tension of the skin. This organized structure is not achieved in wound healing, however, which is why the collagen fibers in scar tissue have a disorganized appearance.

Collagen synthesis is dependent on the presence of vitamin C (ascorbic acid) which acts as a coenzyme. Further cofactors are iron and copper. If there is a deficiency of these substances, satisfactory wound healing is not possible.
Granulation

New highly vascularized tissue grows in from the wound margins in the manner described. Because of its granulated appearance, it is known as granulation tissue and is visible to the naked eye as pin-head-sized rounded nodules of tissue in the wound bed. The granulation tissue is essential for permanent wound closure since it fills out defects and prepares the way for epithelialization.

Figure 7. Granulation Tissue

Pin-head sized, dark red fleshy nodules of tissue on the wound bed make up the new, highly vascularized tissue that fills the defect and creates the preconditions for epithelialization.

The appearance of granulation tissue gives an indication of how the wound will heal. Healthy granulations have a granular appearance, are moist, shiny and hyperemic and have a dark red color. However, if the granulation tissue is smooth, covered with smeary fibrin deposits, soft, pale or shows a bluish discoloration, poor wound healing can be expected.
Fibrinolysis

At the same rate as the fresh, highly vascularized connective tissue develops, the provisional fibrin network is broken down and the closed vessels are recanalized. This breakdown process is known as fibrinolysis and is caused by the enzyme plasmin. Plasmin is present in the blood in the form of an inactive precursor, plasminogen. Plasminogen, like prothrombin, is activated by a number of substances released from damaged cells. The most important of these is tissue plasminogen activator, t-PA.

The activators of fibrinolysis most widely used for therapeutic purposes are streptokinase and urokinase which are administered to dissolve life-threatening blood clots in the coronary arteries of heart attack patients.
**Contraction**

With the formation of new fibers, the mitotic activity of the fibroblasts is concluded. They then transform either into fibroblasts or myofibroblasts. Myofibroblasts, like muscle cells, contain contractile elements which allow them to draw together. The collagen fibers become taut and, as far as possible, aligned to the main contours of tension in the tissue. As a result, the scar tissue shrinks and the functional cutaneous tissue at the wound margin contracts leaving only a small defect.

**Figure 8. The Role of Fibroblasts and Myofibroblasts in Wound Contraction**

*Move your cursor over the cells (fibroblasts and myofibroblasts) in the diagram below to reveal text describing wound contraction.*
Scar Formation

Due to the repair and regeneration processes, the wound margins are bridged over by connective tissue and a scar is formed. At first it is still elevated above the level of the skin and has a reddish color. With time, however, the connective tissue grows tauter and the vascularization decreases. As a result, the scar becomes slightly recessed and turns pale.

Since the pigment-producing cells, the melanocytes, cannot be regenerated, the scar tissue does not turn brown but remains white. Its surface is more or less smooth since the ridged and tessellated pattern of the epidermis is not restored. This tissue contains no hairs and no sebaceous or sweat glands.

Figure 9. Scar Formation

*Move through the following photographs depicting scar formation on a patient's arm by clicking on Next.*

![Site of operation.](image)

This extensive wound after melanoma excision on the thigh cannot undergo primary closure by surgical means because of its extent and depth. It heals by secondary intention and closes mainly by formation of new tissue and contraction. Subsequent coverage by skin grafting was not performed at the patient's request.
Epithelialization

At the end of the wound healing process the surface of the wound is closed by epithelialization. Preconditions for this are an increased rate of cell division in the basal layer of the epidermis and migration of the epithelial cells towards each other from the wound margins until the defect is covered by a fine skin. New epithelial cells are also formed by appendages of the skin, i.e., hair follicles, sebaceous glands and sweat glands.

The mechanisms underlying this cell migration are still largely unknown. Migration requires the presence of a moist substrate, well perfused with blood, as is the case with granulation tissue. If this migratory layer becomes dehydrated, the epithelial cells cannot travel. Recent experiments suggest that the glycoproteins, fibronectin and vitronectin serve as a substrate across which the cells are able to move. However, the way in which epithelial cells are stimulated to migrate in a specific direction and how the reorganization of the cell network necessary for this motion is induced is still unknown.

Epithelial tissue
Remodeling

The process of remodeling is the reorganization of scar tissue. Remodeling is the longest phase of wound healing and can continue for up to 20 years after an injury. It consists mainly of restructuring the collagen fibers which are partially broken down again by enzymes contained in the tissue (collagenases) or newly cross-linked.

Remodeling can be influenced to a certain extent by external factors. For example, the application of compression bandages can reduce scar formation and, thereby, provide a better cosmetic result.
Time Course of Wound Healing

As already mentioned, the individual processes of wound healing do not occur one after the other but overlap to varying degrees.

- The vascular response begins only seconds after the injury, reaches its peak after three to seven days, and then slowly subsides.
- Blood coagulation also begins immediately after the injury. The fibrin network is formed within 24 hours.
- The peak of the inflammatory processes, initiated immediately after the injury, is reached between the third and fifth day. These events are normally complete after 14 days.
- The formation of new connective tissue starts after only 10 to 12 hours and reaches its peak after 6 to 16 days. The mechanical strength of the scar, i.e., its tearing resistance, increases parallel to the collagen content. It reaches its maximum after 12 to 15 days. However, only about 80% of the strength of normal healthy tissue is restored. The scar may take several years to become fully differentiated.
- Epithelialization of superficial wounds occurs within the first 24 hours and is generally complete after 12 days. In the case of deeper, more extensive wounds, however, epithelialization only starts after new connective tissue has formed and takes correspondingly longer.
Recommended Resources

Wound management is a rapidly evolving field. The editors of the UW Wound Academy strongly recommend consulting multiple sources, including the following publications, to continually update your knowledge and verify current approaches to treatment and prevention when making wound management decisions.

Texts


Journals

- Advances in Skin and Wound Care: The International Journal for Prevention and Healing
- Journal of WOCN (Wound Ostomy and Continence Nursing)
- OWM -- Ostomy Wound Management
- Today's Wound Clinic
- Wounds: A Compendium of Clinical Research and Practice

References

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