HOW DOES THE SKIN HEAL WOUNDS?

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Wounds are an unavoidable part of our life. Some heal fast, some take time, some leave scars behind. An organ in the human body with the inherent ability to heal itself is the skin. How does the skin do this?

Do you know someone who has never been injured? No? Here's a simpler question — do you remember how many times you've had a cut or a wound? Doesn't it seem unlikely that anyone would answer these questions with a yes?!

In medical terms, a wound is defined as an injury to the body that involves the breaking of a protective membrane (such as the skin) and damage to underlying tissues. Cuts and wounds are an everyday occurrence, which left unchecked can reduce tissue integrity, resulting in loss of function and compromised survival.

Millions of years of evolution have equipped our bodies with self-healing mechanisms. This is why we rarely worry about minor injuries. Even the medical care of major wounds (like those from burns or accidents) has historically focused on the development of techniques that supplement the body's built-in healing mechanisms (see **Box 1**). To do this, we are constantly attempting to uncover the specific cells and molecular factors involved in these mechanisms. One organ that has been studied most extensively in this regard is the skin.

The human skin

As the largest organ of the body in terms of its surface area and weight, the skin acts as a protective barrier against pathogens, dehydration, chemical toxins, and harmful UV rays. With its immense regenerative potential, the wound repair capacity of the skin is among the highest of all the organs in the body, exceeding even that of the heart (after a heart attack) and the brain (after a stroke).

Mammalian skin is composed of three layers (see **Fig. 1**):

- **The epidermis:** is the visible portion of the skin. It consists of cells called **keratinocytes**, which are organized into different sub-layers. Epidermal stem cells in the innermost sub-layer divide to populate other layers. As the dividing cells mature, they get pushed upwards to replace worn out/damaged cells on the surface. The epidermis also consists of hair follicles, sweat glands, and sebaceous glands.
- **The dermis:** forms the support structure below the epidermis. It mostly contains cells called **fibroblasts,** which produce the extracellular matrix (ECM). Most blood vessels and nerves lie in this layer and have extensions connecting them to cells in the epidermis.
- **The hypodermis:** is made up of adipose or fat tissue. Its presence is vital in providing insulation to the body.

Cells populating all three skin layers drive the healing process. In addition, the skin has some immune cells, like macrophages and mast cells, which are capable of an immediate response to injury or infection (see **Box 2**).

Wound healing in the skin

The healing process occurs in four phases over a period ranging from seconds to months (see **Fig. 2**).

(a) Hemostasis

The first step in wound healing involves the formation of a clot to limit the loss of blood.

A clot is formed by the coordinated activity of many cells and tissues — each of which receives information on when and how to act through multiple signaling events. In intact blood vessels, endothelial cells (which form the inner lining of blood vessels) release chemicals (like prostacyclin) to prevent the accumulation of platelets and ensure smooth blood flow. When wounding results in damage to blood vessels, this inhibition is no longer effective and platelets quickly accumulate at the site of injury. These platelets secrete copious amounts of a fiber-like protein, called **fibrin,** that interconnect to form an insoluble mesh (**clot**) at the wound site, thereby sealing it. Hemostasis typically occurs within a few seconds to a few minutes.

Simultaneously, wound healing is initiated by the release of chemical triggers from a variety of sources. Healthy neighboring cells are informed of the injury through two kinds of molecular signals. One signal consists of molecular motifs, called **Damage Associated Molecular Patterns** (DAMPs), which are characteristic of the cellular debris (like, DNA, RNA, proteins) of dead and damaged body cells at the wound site. The other consists of molecular motifs, called **Pathogen Associated Molecular Patterns** (PAMPs), that are typical of metabolites of resident and pathogenic bacteria (like membrane lipopolysaccharides, peptidoglycans

Box 1. A brief history of wound care:

Two methods of supplementing healing that have been popular through the ages are the covering of wounds and the use of disinfectants. The oldest known record of wound care is that on a Mesopotamian clay tablet from ~2200 BC. This tablet describes 'three healing gestures' — cleaning a wound with beer; preparing a dressing out of oil and plant extracts with mud or clay; and, finally, wrapping the wound with a bandage soaked in wine and turpentine (an oil commonly used to remove paint nowadays). *Papyruses* dating back to 1400 BC suggest that the Egyptians used honey and adhesive bandages to cover their wounds. In the 5th century BC, Hippocrates recommended that injuries should be cleansed with vinegar or wine, and bandaged with wool soaked in wine.

Over the centuries, other aids to supplement healing were developed. Records of Heron of Alexandria mention the extensive use of a syringe called ***Pyulkos*** (meaning "pus-puller" in Greek) in 280 BC to inject medicinal extracts and suck pus out of deep wounds. Yet, its usefulness was forgotten in the Western world until its rediscovery nearly 2000 years later! Two military surgeons, Pare and Larrey, suggested maggot infestation as a method for disinfecting wounds. Medicinal leeches (*Hirudo medicinalis*) were also an indispensable part of medical practice in the 19th century. Interestingly, surgery became a reliable method for wound care only in the latter half of the 19th century, with the use of carbolic acid as an antiseptic by Joseph Lister in 1865.

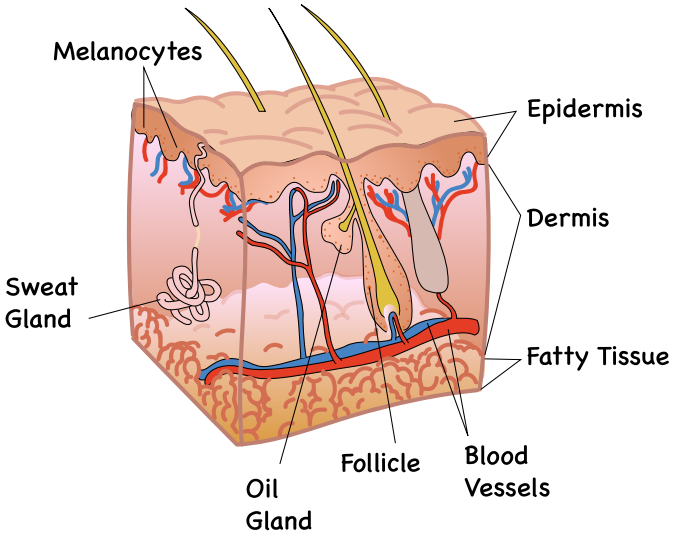

Fig. 1. The three layers of human skin.

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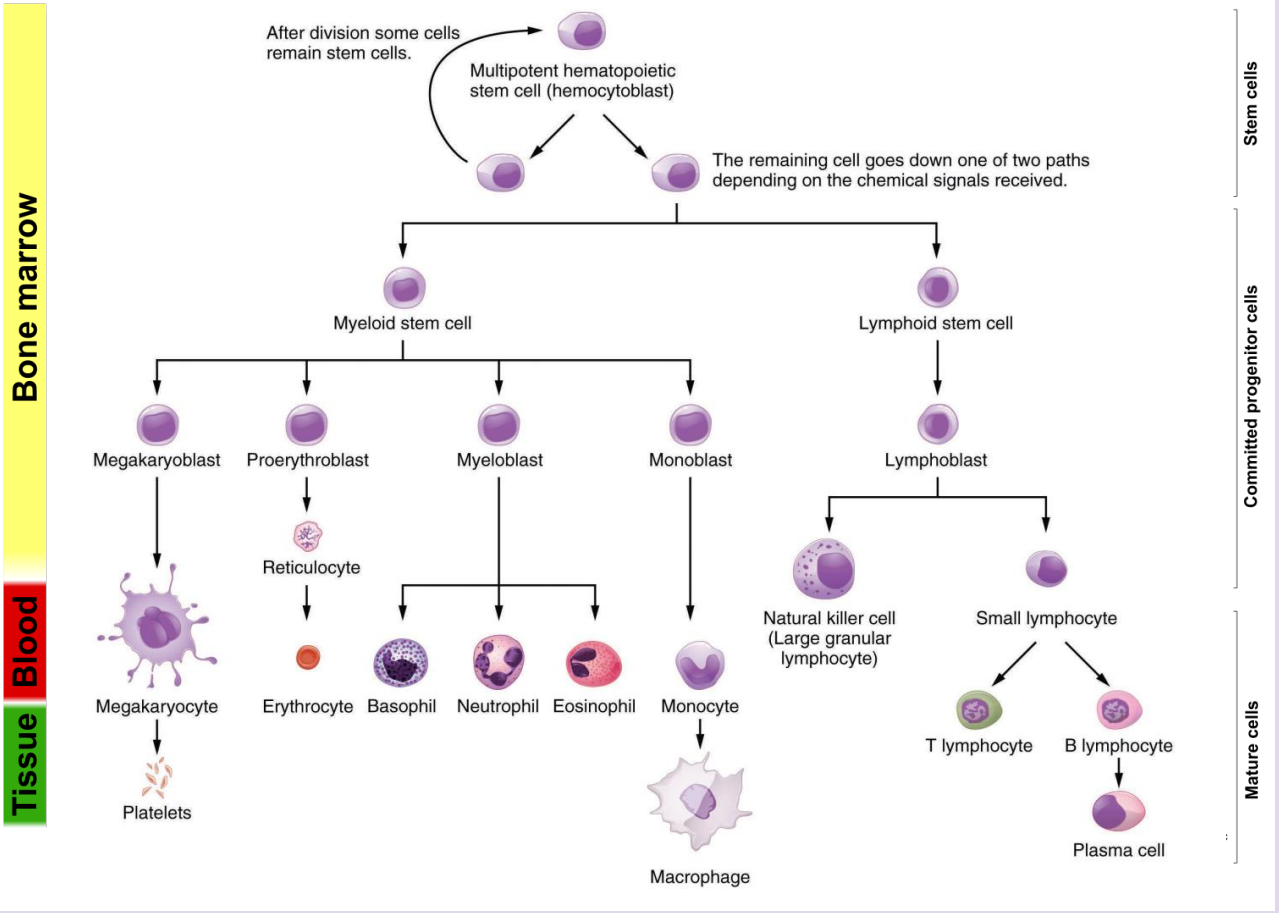
etc.) that invade the body through an open wound. On recognizing these DAMPs and/or PAMPs, a cascade of chemical events is activated in healthy neighbouring cells. This cascade results in the secretion of chemicals that attract immune cells (like macrophages) to the wound site. Recent studies suggest that a release in cellular tension (because of the loss of cell-cell bonding in the damaged tissues) can also act as a trigger for the initiation of wound healing.

The platelets that form the clot at the wound site also trigger healing by releasing chemical signals, such as platelet derived growth factor (PDGF). Cells of the immune system (like macrophages, monocytes, neutrophils) and connective tissue (like fibroblasts) recognize these chemical signals and migrate towards the wound site. These

Box 2. A brief introduction to immune cells:

Immune cells are like the soldiers of our body since they protect us from harmful invaders. They originate from a particular kind of pluripotent stem cell, called **hematopoietic stem cell**, in the bone marrow. This cell, in turn, gives rise to two kinds of cells — called **myeloid progenitor** and **lymphoid progenitor**.



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The myeloid progenitor gives rise to four categories of more specialized cells. Megakaryocytes form platelets for blood clot formation. Erythrocytes or red blood cells make up the oxygen transport system of the body. Granulocytes (with their numerous granules) and mature monocytes (called **macrophages**) form the innate immune system, acting as the first responders to any infectious pathogen.

The lymphoid progenitors mature into **T-** and **B-cell precursors** (called so because they mature in the thymus and bone marrow respectively). Lymphocytes form the adaptive immune system, which comes into play in the later stages of an injury or infection. T cells, along with dendritic cells and Natural Killer (NK) cells, kill infected cells of the body. B cells are specialized to produce antibodies, which coat the infected cells and enable their recognition by T cells and NK cells. Some of these specialized antibodies also remain in the body and act like natural vaccines, helping us launch a faster immune response against the same invaders in future.

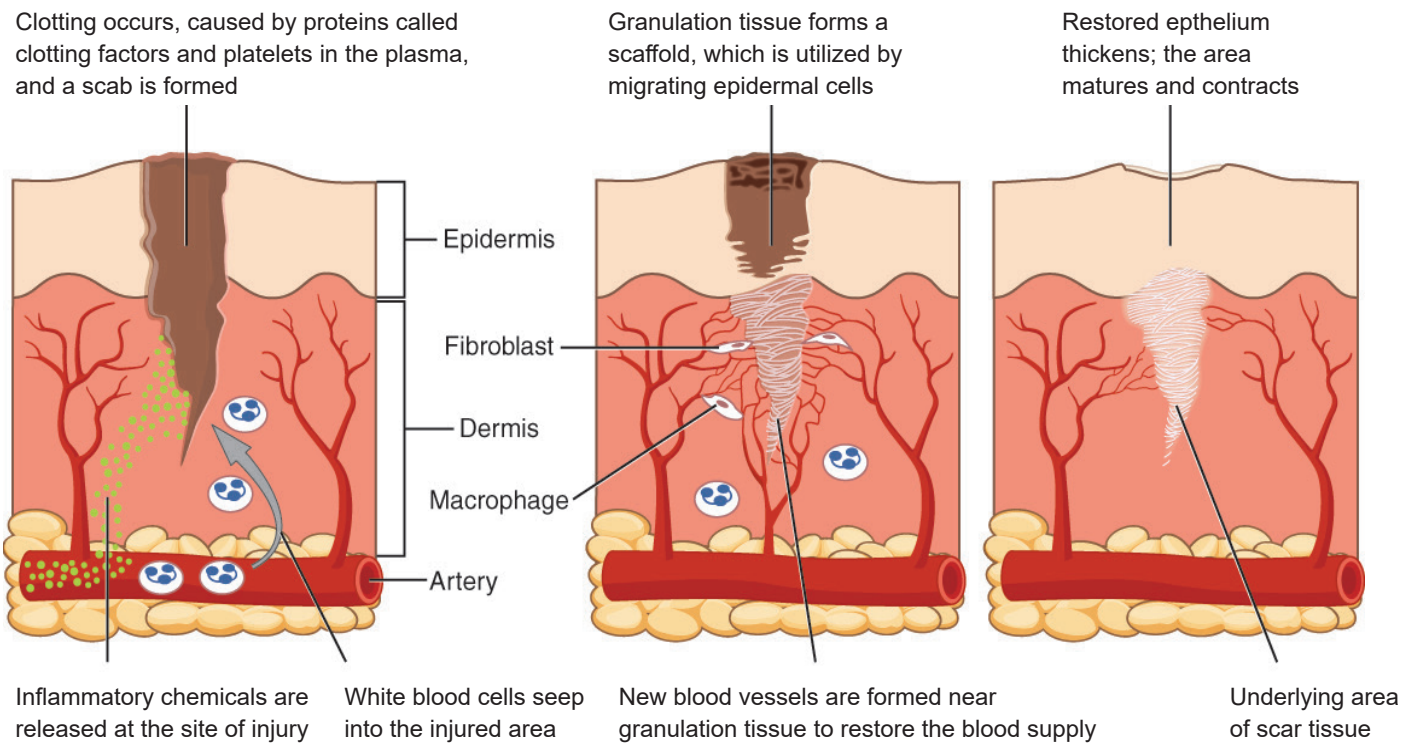

Fig. 2. Stages of wound healing.

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infiltrating immune cells perform two roles – they fight infectious pathogens that enter the body through this breach in its barrier, and remove debris accumulated in the form of dead cells.

(b) Inflammation

Inflammation can be described as a local response of the immune system to injury. In the 1st century AD, Aulus Celsus (a Roman encyclopaedist, known for his extensive work in the field of medicine) described the four fundamental signs of inflammation – *calor* (heat), *dolor* (pain), *rubor* (redness) and *tumor* (swelling). Even today, early phases of healing are characterized by these signs.

Inflammation of the skin usually appears within a few hours of the injury and may persist for a period ranging from a few (2-4) days to a couple of weeks. Damaged skin cells secrete cellular chemicals, which induce healthy cells in the vicinity to proliferate and migrate to the site of injury. Some of these chemicals diffuse into surrounding tissue, attracting and activating immune cells in the skin and blood vessels. For e.g., mast cells and basophils release histamine (a cellular signaling molecule, often the cause of itching during an allergic response) that causes blood vessels to dilate and become more permeable. This promotes the leakage of various immune cells and plasma proteins (like albumin and antibodies) from blood into the damaged tissue.

Immune cells enter the wound site in phases, forming an army to fight off any microbial invaders along its borders. The first cells to do this are components of the innate immune system. For e.g., macrophages and neutrophils help clean-up debris from pathogens and body cells. Macrophages also secrete cytokines to attract dermal fibroblasts to the wound site (see **Box 3**). These cytokines help form new blood vessels in a process called **angiogenesis** to replace damaged ones. The innate immune response is followed by an adaptive one involving the T and B lymphocytes. In addition, all the immune cells at the wound site secrete chemical signals (factors) that activate epidermal keratinocytes, dermal fibroblasts, and skin stem cells (which

Box 3. Cytokines and growth factors:

Cells release chemicals called cytokines. The term 'cytokine' is derived from a combination of two Greek words – '*cyto*' meaning cell, and '*kinos*' meaning movement. These chemicals aid cell-to-cell communication in immune responses and stimulate the movement of cells towards sites of inflammation. Some of these, categorized as interferons, are released against viral infections (e.g., INFγ). Others, called **interleukins**, promote inflammation (e.g., IL1).

Growth factors are a class of cytokines that, as their name suggests, signal growth and proliferation (e.g., TGFβ1, PDGF, and FGF). These are produced in response to developmental and regenerative signals.

lie in hair follicles as well as other parts of the epidermis). Cross talk, through chemical signaling, between resident and infiltrating cells (immune cells, keratinocytes, fibroblasts, and stem cells) at the wound site leads to the next phase of the healing program.

(c) Proliferation

Once activated, fibroblasts proliferate and migrate towards the wound site, where they secrete components of the ECM. The ECM includes proteins like collagen, fibronectin and other compounds that are necessary to form a supporting structure for the closure of the wound. Because of their ability to extend and retract, myofibroblasts (also known as **activated fibroblasts**) mediate contraction of the wound area through a process involving communication with the ECM.

The immune cells and fibroblasts secrete cytokines that activate epidermal stem cells. These factors include transforming growth factor (TGF-β), keratinocyte growth factor (KGF), and epidermal growth factor (EGF). Once activated, epidermal and hair follicle stem cells proliferate and migrate towards and over the newly formed ECM scaffold to seal the wound. This process, known as **reepithelialization**, culminates in wound-closure.

The lack of oxygen at the injury site triggers resident cells to release chemical signals to initiate angiogenesis. Macrophages and fibroblasts are known to promote this process by releasing chemicals like lactic acid, biogenic amines, fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF). This process is a prerequisite for renewed oxygen supply to the newly formed tissue.

(d) Remodeling

The activation of fibroblasts during the proliferative phase results in the deposition of excessive amounts of ECM proteins (e.g., collagen) in the skin. This is responsible for the formation of scars. Severe wounding leads to the activation of a greater number of fibroblasts, resulting in scars that are much deeper and stay for much longer.

One hallmark of this phase is the activation of protein degrading enzymes, like the Matrix Metalloproteinases (MMPs). MMPs chew up excessive ECM proteins, making space for growing blood vessels and newly formed cells. The action of this enzyme is apparent in the fading of scar tissue, a process that can take anywhere between a few months to many years to accomplish, depending on the severity of the wound. For e.g., you may have observed the scar from a paper cut disappearing in a few days, while that from a deeper wound (e.g., from a fall on the road) taking months or years to completely vanish.

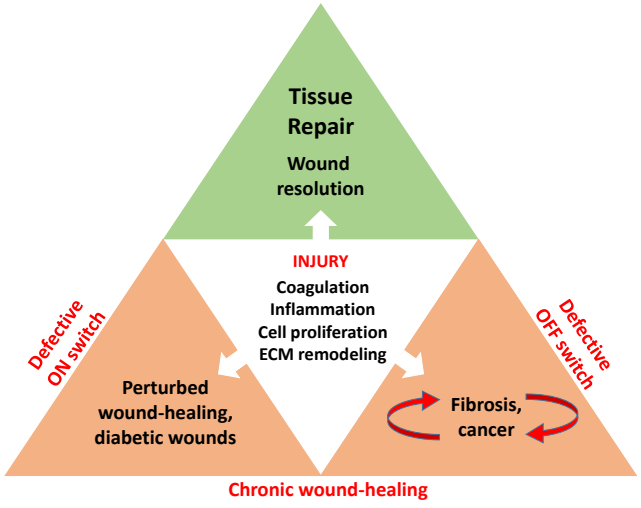

Fig. 3. Wound healing is not an error-free process.

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To conclude

Today, we have more than 5000 products to aid wound healing, and the future holds immense promise. Wound-dressing material with the capacity for sustained release of various growth factors (chemicals that stimulate cell division and migration) and disinfectants (antiseptic chemicals to avoid infection) are currently under development to enhance the wound healing process.

Wound healing is, however, not an error-free process (see Fig. 3). In some cases, healing is not initiated because some cellular actors are unresponsive to wound signals. One example of this is seen in non-healing diabetic ulcers — high blood sugar interferes with both the immune response and the potential of skin cells to proliferate and migrate. Wound healing can also be delayed with age because of the reduced production of growth factors at the wound site. Both cases are treated by providing growth factors that mimic the natural wound-response of cells. This kick-starts the healing program and helps in re-epithelialization.

In a healthy body, as a wound heals, the cells that collect at the site of injury return to their normal functions (**homeostasis**). However, in some cases, the wound-healing response continues even when not needed. Research reveals that severe scar formation can hinder the ability of the wounded tissue to function normally (see **Box 4**). In such cases, excessive secretion of collagen from hyperactive fibroblasts results in the development of a pathological condition called **fibrosis**. Fibrosis can occur in connective tissues of various organs in our body, such as the heart, liver, kidneys, lungs etc. This condition leads to loss of structure and function of the organ involved. In severe cases,

it can even lead to death. Skin fibrosis is manifested in several forms such as keloids, scleroderma, and various types of skin cancers.

Interestingly, the proteins and processes (like increased inflammation, angiogenesis, and fibroblast activation) that are central to the wound healing process are found to be exaggerated in certain classes of cancer. This discovery has led to the popular hypothesis that describes cancer as an over-healing wound. Parallel research on wound-healing and cancer may hold the potential to open up new therapeutic targets for both.

Box 4. Skin scarring:

In order to perform its functions efficiently, the skin has a supply of stem-cells, blood vessels, nerves, hair, and sweat glands. These components of the skin get activated upon injury and help the skin heal efficiently. However, it is difficult for the specialized structure of the skin to completely regain its native state (**regeneration**). The evolutionary advantage of rapid healing minimizes the chances of infection and fluid loss, but it is achieved at the cost of complete regeneration! Thus, wound-healing often results in the formation of scar tissue that lacks the strength and flexibility of healthy skin.

Key takeaways

- Being the outermost protective covering of our body, the skin has evolved to develop an immense self-repair/healing capacity.
- Wound healing in skin involves four stages — hemostasis, inflammation, proliferation and remodeling.
- Hemostasis is marked by the formation of blood clots — a quick response to stop blood loss.
- During inflammation, immune cells enter the wound site to fight against infection
- The proliferation phase involves the division of activated cells and their migration to the wound site to cover the injury.
- During remodeling, the excessive deposition of the extracellular matrix, which is visible as a scar, gets resolved.
- Diabetes can cause delayed initiation of the wound response, whereas over-healing of wounds can give rise to fibrosis/cancer.

Note: Image used in the background of the article title — Human skin. Credits: Kowshik Kuri. URL: https://www.flickr.com/photos/kowshikkuri/26739953088/in/photostream/. License: CC-BY.

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