Fundamentals of Acute Wound Healing

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Abstract

Acute wound healing is a complex process activated by damage to body tissue. The process occurs in a series of well-coordinated bur overlapping stages occurring in a temporal manner. Firstly, haemostasis occurs immediately after wounding to prevent excessive blood loss. Shortly after, inflammatory processes including the complement system are activated to protect the wound from foreign particles and bacteria, and also to accelerate the wound healing process. During proliferation, fibroblasts contribute to the formation of the new extracellular matrix and new epithelial tissue. Finally, long-term collagen remodeling occurs in an attempt to normalize skin integrity. The article reviews the normal wound healing process and the underlying key molecular and cellular mechanisms.

Introduction

In order to understand pathological conditions that are due to abnormal wound healing processes (i.e., fibrosis and chronic ulceration), one must first comprehend the physiological process of wound healing. Any injury resulting in tissue damage in the human body activates an acute healing response. The acute wound healing process comprises a series of coordinated cellular processes carried out in a controlled manner.[1]

Acute wound healing occurs in four well-characterised and overlapping stages (Figure 1):
1. Haemostasis
2. Inflammation
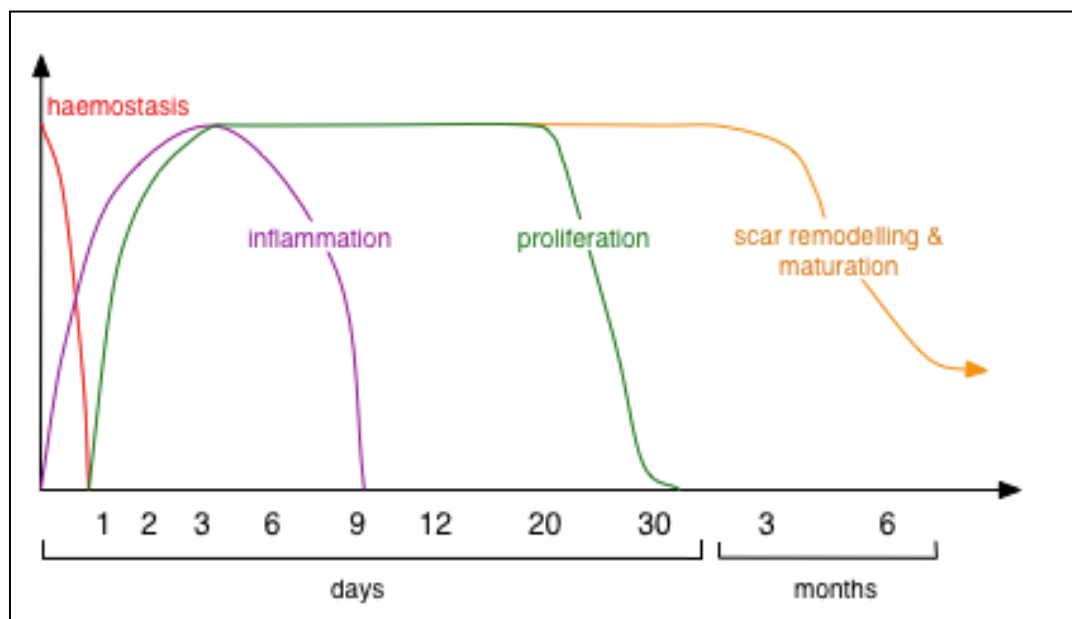3. Proliferation
4. Remodeling & Scar Maturation

Figure 1: The Stages of Wound Healing. Haemostasis starts immediately after a wound has been created. Inflammation occurs from about 15 mins to 9 days, and proliferation is active from day 3 onwards and can last for up to a month. Scar remodeling and maturation continues for months after the wound has been created.[2]

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Haemostasis (Immediate)

Clinical signs: blanching, formation of clot.

Damage to the microvasculature of a tissue results in extravasation of blood into the site of injury. Meanwhile, the following processes are triggered to achieve haemostasis:[2]

- local vasculature constricts to reduce further blood loss
- a coagulation cascade is triggered that leads to the formation of thrombin.

Thrombin catalyses the conversion of fibrinogen to fibrin. Activated fibrin monomers are polymerized by covalent cross-linkage by Factor XIII. Fibrin polymers bind to platelets to form the haemostatic plug, which appears as a clot.[3]

Inflammation (15 min – day 6)

Clinical signs: erythema, pain, swelling and heat (the well characterized rubor, dolor, tumor and calor stigmata as identified by the ancient Greek physician Galen)

Inflammation occurs in two phases – the early phase and the late phase.

Early phase (days 1-2)

Vasodilation and increase in the permeability of capillaries promote the movement of inflammatory proteins and cells into the wound from the intravascular compartment. Mast cells produce histamine and leukotrienes which cause vasodilation. Clotting products such as kinins and thrombin also enhance capillary permeability.[1,4]

The complement system is also activated in the early inflammatory phase. Complement factors C3a and C5a are chemoattractants which attract inflammatory cells (e.g. macrophages) into the wound. Growth factors (e.g. PDGF), cytokines (e.g. IL-8) and chemokines (e.g. GRO-α/CXCL1) attract polymorphonuclear cells (PMNs), which move to the site of injury by adhering to the vascular endothelium (margination) and traversing the vessel wall (diapedesis). PMNs are able to phagocytose foreign particles and bacteria, and are also capable of secreting other cytokines, including tumour necrosis factor alpha (TNF-α).[5,6]

Late phase (days 2-3)

Monocytes transform into macrophages upon arrival at the site by a number of chemoattractants including fibronectin, elastin, complement factors andgrowth factors (PDGF, VEGF, TGF-β). Macrophages are in turn capable of phagocytosis and the production, storage and release of pro-inflammatory factors. Macrophages produce cytokines such as certain interleukins and TNF-α that drive the inflammatory process. They also stimulate angiogenesis and collagen production by fibroblasts.[5]

T lymphocytes regulate the wound healing process, albeit not in a straightforward fashion. Depletion of CD4 and CD8 cells alter wound strength, whilst dendritic γδ epidermal T cells (DETCs) release factors which stimulate the growth of keratinocytes and fibroblasts.[7]

Proliferation (day 3 to week 2)

Clinical signs: pink, soft and granular appearance (granular tissue)

During the proliferation phase, fibroblasts migrate to the wound, the temporary haemostatic plug is replaced by new extracellular matrix and granulation tissue is deposited.

Fibroblast migration

A number of factors (TGF-β, PDGF, fibronectin, etc.) attract fibroblasts to the site of injury. Fibroblasts are present nearby and can also be derived from mesenchymal cells. Fibroblasts produce proteins and attract other factors which are required for the formation of the extracellular matrix, such as fibrin, fibronectin, and hyaluronic acid. Fibronectin provides a scaffold to which other fibroblasts can bind. Later, fibroblasts also release proteoglycans and collagen which are crucial for ECM formation.

Formation of the extracellular matrix

The extracellular matrix (ECM) comprises fibrous structural proteins (collagens, elastin) and adhesive glycoproteins.[8]
Function of ECM:

Collagens

Over 20 types of collagens exist, but Type I collagen is the predominant collagen (70%) in wound healing, followed by Type III collagen (30%), and they are produced by fibroblasts.[2]
Collagens give strength to the wound and also allow the activity of other cells vital to wound healing.
The hydroxylation of proline and lysine residues at the endoplasmic reticulum during the intracellular production of collagen is an important step, as it allows collagen polypeptides which are alpha-chains to assume a triple helical structure capable of fibril aggregation. These collagen bundles form the bulk of connective tissue in a healing wound.[5,9]

Other collagens, such as type IV collagen, do not form fibril bundles and instead form part of the basement membrane.

The Role of other proteins in the wound healing process:

Fibronectin and integrins are adhesive glycoproteins that bind to various components of the ECM which are capable of mediating cell signal pathways, cell migration and cell growth in the wound.[8]

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Proteoglycans are polypeptides to which various glycosaminoglycans (GAGs) such as hyaluronic acid and heparan sulphate attach. They regulate molecular and cellular activities within the ECM. Hyaluronic acid has the ability to modify adhesions between collagen and cells, thereby permitting the migration of other cells in the ECM. Chondroitin and dermatan sulphates facilitate the process of collagen fibril aggregation.[1]

Granulation (3-5 days) and Angiogenesis

Granulation tissue appears pink, soft and granular (beneath a scab)
The histological appearance of granulation tissue is that of fibroblasts, collagen and formation of new blood vessels.[1,2]

Angiogenesis

•endothelial cells migrate towards the site of injury to replace damaged vasculature, driven by pro-angiogenic factors.
•Pro-angiogenic factors: low pH, increase in lactate, hypoxia, growth factors and cytokines such as VEGF, PDGF, FGF, angiopoietin and TGF-beta.
Endothelial cells express integrins and MMPs to help them move around the ECM.

Re-epithelialization

Keratinocytes from the wound margin migrate to form a new layer of epidermis covering the wound. Migrating and proliferating keratinocytes first forms a new basement membrane and then differentiates giving rise to the stratified epithelium.
Keratinocyte migration and proliferation is stimulated by a number of growth factors and cytokines such as epidermal growth factor (EGF) and nerve growth factor (NGF). The environment in the wound also plays a role in facilitating keratinocyte movement and growth. Re-epithelialization can be completed within two days in shallow wounds.[8,10]

Remodelling & Maturation

Clinical appearance: wound contraction, normalization of skin thickness, and reduced redness.
Remodeling and maturation of the wound starts during the proliferative phase but continues for a long time after injury (up to many months). Key components of wound healing mentioned before such as fibroblasts, collagen and blood vessels also contribute to this phase of wound healing.
Another family of enzymes, matrix metalloproteinases (MMPs), is responsible for breaking down collagen during remodeling of the ECM. Collagen degradation by MMPs is regulated by factors such as TGF-β and PDGF. A balance of collagen synthesis and degradation is achieved after 3 weeks, where type I collagen in the ECM increases in proportion to the other collagen types and proteogylcans.[1]

Eventually, new vasculature stops growing, inflammatory cells reduce in numbers, and the wound contracts about 5 days after the initiation of the wound healing process for a couple of weeks. The granulation tissue becomes a scar made up of fibrous tissue, collagen, elastic tissue, and components of the ECM.[2]

Excessive wound healing

Excess scar tissue is laid down due to overproduction of many components of the wound healing process, two types of which are discussed here.

Hypertrophic scars

Clinical appearance: wounds are itchy, red and raised, and at a later stage pale and flat.
In hypertrophic scars, excess collagen leads to the formation of a scar which is raised. The risk factors for the formation of hypertrophic scars are:[2]
Scars lying across lines of skin tension
Areas of excessive skin movement
Deep skin burns
Wounds left to granulate

Keloid scars

Clinical appearance: scar that has grown beyond original wound, raised, red initially but pale as time goes on. May persist and grow for many months.
Keloid scars are more aggressive than hypertrophic scars in that the scar extends beyond the original wound into normal tissue. Collagen synthesis as compared to hypertrophic scars is increased 3-fold, and 20-fold compared to normal skin. The composition of cross-linked, mature collagen is also lower in keloids.[2,11]
They are more common in people of Afro-American ethnicity, and familial predisposition seems to play a significant role.

Scarless wound healing

Scarless wound healing occurs in the human foetus, and also in areas of the body such as the oral mucosa. Essentially, scarless wound healing progresses through similar stages of cutaneous wound healing, except for the absence of scarring. There have been several hypotheses of how the processes differ; the lack of scar formation in the oral mucosa can be due to altered or dampened inflammation, differential modulation by fibroblasts, or the presence of stem cells that allow replacement of new cells.[12]
The understanding of scarless wound healing would have clinical implications such as in tissue engineered skin substitutes that is currently being explored.

Conclusion

The wound healing process is a complex and sophisticated phenomenon. A sound understanding of this dynamic process is essential in order to understand pathological conditions attributable to abnormal wound healing. Future novel therapeutics aim to harness the natural scarless wound healing process that is observed in certain scenarios. Further research in this fascinating area is currently ongoing with pioneers in the field making promising advances.

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