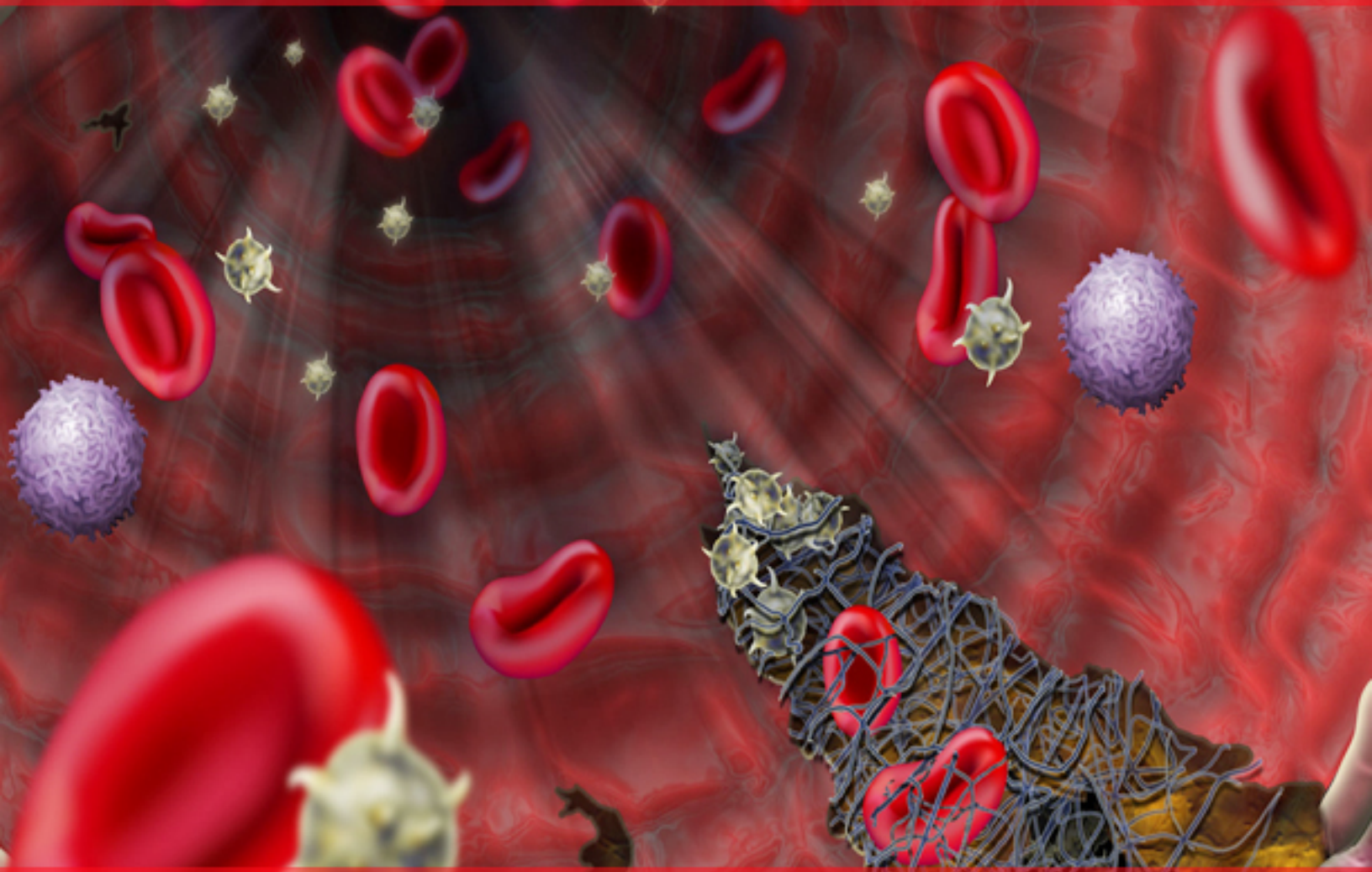
Wound Healing



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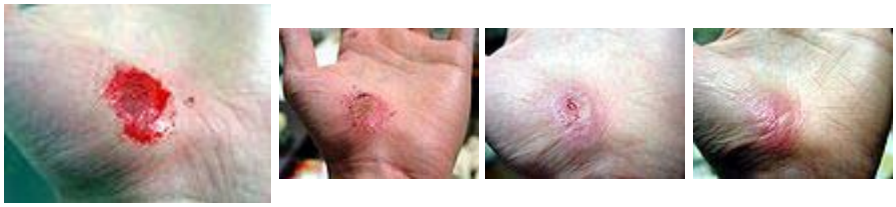
Wound Healing

Hand abrasion



Approximate days since injury

0 2 17 30

Wound healing, or **wound repair**, is an intricate process in which the skin (or another organ-tissue) repairs itself after injury. In normal skin, the epidermis (outermost layer) and dermis (inner or deeper layer) exists in a steady-state equilibrium, forming a protective barrier against the external environment. Once the protective barrier is broken, the normal (physiologic) process of wound healing is immediately set in motion. The classic model of wound healing is divided into three or four sequential, yet overlapping, phases: (1) hemostasis, (2) inflammatory, (3) proliferative and (4) remodeling. Upon injury to the skin, a set of complex biochemical events takes place in a closely orchestrated cascade to repair the damage. Within minutes post-injury, platelets (thrombocytes) aggregate at the injury site to form a fibrin clot. This clot acts to control active bleeding (hemostasis).

In the inflammatory phase, bacteria and debris are phagocytosed and removed, and factors are released that cause the migration and division of cells involved in the proliferative phase.

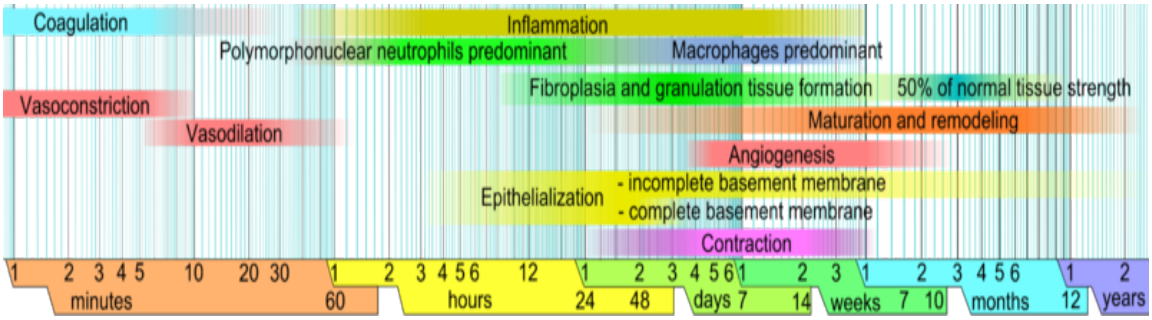
The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction. In angiogenesis, new blood vessels are formed by vascular endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts grow and form a new, provisional extracellular matrix (ECM) by excreting collagen and fibronectin. Concurrently, re-epithelialization of the epidermis

occurs, in which epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue.

In contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. When the cells' roles are close to complete, unneeded cells undergo apoptosis.

In the maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis.

However, this process is not only complex but fragile, and susceptible to interruption or failure leading to the formation of chronic non-healing wounds. Factors which may contribute to this include diabetes, venous or arterial disease, old age, and infection.



Approximate times of the different phases of wound healing, with faded intervals marking substantial variation, depending mainly on wound size and healing conditions.

Early vs cellular phase

As mentioned above, wound healing is classically divided into hemostasis, inflammation, proliferation, and remodeling. Although a useful construct, this model employs considerable overlapping among individual phases. Recently, a complementary model has been described, such that the many elements of wound healing are more-clearly delineated. The importance of this new model becomes more apparent through its utility in the fields of regenerative medicine and tissue engineering. In this construct, the process of wound healing is divided into major two phases: *early phase* and *cellular phase*:

The early phase, which begins immediately following skin injury, involves cascading molecular and cellular events leading to hemostasis and formation of an early, makeshift extracellular matrix—providing structural support for cellular attachment and subsequent cellular proliferation.

The cellular phase follows the early phase, and involves several types of cells working together to mount an inflammatory response, synthesize granulation tissue, and restore the epithelial layer. Subdivisions of the cellular phase are: Macrophages and

inflammatory components (within 1–2 days), Epithelial-mesenchymal interaction: re-epithelialization (phenotype change within hours, migration begins on day 1 or 2, Fibroblasts and myofibroblasts: progressive alignment, collagen production, and matrix contraction (between day 4 day 14), Endothelial cells and angiogenesis (begins on day 4), Dermal matrix: elements of fabrication (begins on day 4, lasting 2 weeks) and alteration (begins after week 2, lasting weeks to months—depending on wound size.).

Inflammatory phase

Just before the inflammatory phase is initiated, the clotting cascade takes place in order to obtain hemostasis, or stop blood loss by way of a fibrin clot. Thereafter, various soluble factors (including chemokines and cytokines) are released to attract cells that phagocytise debris, bacteria, and damaged tissue, in addition to releasing signaling molecules that initiate the proliferative phase of wound healing.

Clotting cascade

When tissue is first wounded, blood comes in contact with collagen, triggering blood platelets to begin secreting inflammatory factors. Platelets also express glycoproteins on their cell membranes that allow them to stick to one another and to aggregate, forming a mass.

Fibrin and fibronectin cross-link together and form a plug that traps proteins and particles and prevents further blood loss. This fibrin-fibronectin plug is also the main structural support for the wound until collagen is deposited. Migratory cells use this plug as a matrix to crawl across, and platelets adhere to it and secrete factors. The clot is eventually lysed and replaced with granulation tissue and then later with collagen.

Platelets, the cells present in the highest numbers shortly after a wound occurs, release a number of things into the blood, including ECM proteins and cytokines, including growth factors. Growth factors stimulate cells to speed their rate of division. Platelets also release other proinflammatory factors like serotonin, bradykinin, prostaglandins, prostacyclins, thromboxane, and histamine, which serve a number of purposes, including to increase cell proliferation and migration to the area and to cause blood vessels to become dilated and porous.

Vasoconstriction and vasodilation

Immediately after a blood vessel is breached, ruptured cell membranes release inflammatory factors like thromboxanes and prostaglandins that cause the vessel to spasm to prevent blood loss and to collect inflammatory cells and factors in the area. This vasoconstriction lasts five to ten minutes and is followed by vasodilation, a widening of blood vessels, which peaks at about 20 minutes post-wounding. Vasodilation is the result of factors released by platelets and other cells. The main factor involved in causing vasodilation is histamine. Histamine also causes blood vessels to become porous, allowing the tissue to become edematous because proteins from the bloodstream leak into

the extravascular space, which increases its osmolar load and draws water into the area. Increased porosity of blood vessels also facilitates the entry of inflammatory cells like leukocytes into the wound site from the bloodstream.

Polymorphonuclear neutrophils

Within an hour of wounding, polymorphonuclear neutrophils (PMNs) arrive at the wound site and become the predominant cells in the wound for the first two days after the injury occurs, with especially high numbers on the second day. They are attracted to the site by fibronectin, growth factors, and substances such as kinins. Neutrophils phagocytise debris and bacteria and also kill bacteria by releasing free radicals in what is called a 'respiratory burst'. They also cleanse the wound by secreting proteases that break down damaged tissue. Neutrophils usually undergo apoptosis once they have completed their tasks and are engulfed and degraded by macrophages.

Other leukocytes to enter the area include helper T cells, which secrete cytokines to cause more T cells to divide and to increase inflammation and enhance vasodilation and vessel permeability. T cells also increase the activity of macrophages.

Macrophages

Macrophages are essential to wound healing. They replace PMNs as the predominant cells in the wound by two days after injury. Attracted to the wound site by growth factors released by platelets and other cells, monocytes from the bloodstream enter the area through blood vessel walls. Numbers of monocytes in the wound peak one to one and a half days after the injury occurs. Once they are in the wound site, monocytes mature into macrophages. The spleen contains half the body's monocytes in reserve ready to be deployed to injured tissue.

The macrophage's main role is to phagocytize bacteria and damaged tissue, and they also debride damaged tissue by releasing proteases. Macrophages also secrete a number of factors such as growth factors and other cytokines, especially during the third and fourth post-wounding days. These factors attract cells involved in the proliferation stage of healing to the area., although they may restrain the contraction phase. Macrophages are stimulated by the low oxygen content of their surroundings to produce factors that induce and speed angiogenesis. and they also stimulate cells that reepithelialize the wound, create granulation tissue, and lay down a new extracellular matrix. By secreting these factors, macrophages contribute to pushing the wound healing process into the next phase.

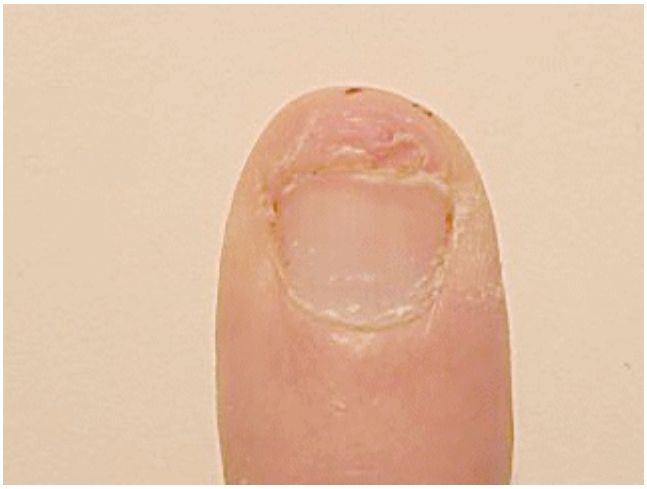
Decline of inflammatory phase

As inflammation dies down, fewer inflammatory factors are secreted, existing ones are broken down, and numbers of neutrophils and macrophages are reduced at the wound site. These changes indicate that the inflammatory phase is ending and the proliferative phase is underway. In vitro evidence, obtained using the dermal equivalent model,

suggests that the presence of macrophages actually delays wound contraction and thus the disappearance of macrophages from the wound may be essential for subsequent phases to occur.

Because inflammation plays roles in fighting infection, clearing debris and inducing the proliferation phase, it is a necessary part of healing. However, inflammation can lead to tissue damage if it lasts too long. Thus the reduction of inflammation is frequently a goal in therapeutic settings. Inflammation lasts as long as there is debris in the wound. Thus the presence of dirt or other objects can extend the inflammatory phase for too long, leading to a chronic wound.

Proliferative phase



2 months of healing on a human finger after injury.

About two or three days after the wound occurs, fibroblasts begin to enter the wound site, marking the onset of the proliferative phase even before the inflammatory phase has ended. As in the other phases of wound healing, steps in the proliferative phase do not occur in a series but rather partially overlap in time.

Angiogenesis

Also called neovascularization, the process of angiogenesis occurs concurrently with fibroblast proliferation when endothelial cells migrate to the area of the wound. Because the activity of fibroblasts and epithelial cells requires oxygen and nutrients, angiogenesis is imperative for other stages in wound healing, like epidermal and fibroblast migration. The tissue in which angiogenesis has occurred typically looks red (is erythematous) due to the presence of capillaries.

Stem cells of endothelial cells, originating from parts of uninjured blood vessels, develop pseudopodia and push through the ECM into the wound site to establish new blood vessels.

Endothelial cells are attracted to the wound area by fibronectin found on the fibrin scab and chemotactically by angiogenic factors released by other cells, e.g. from macrophages and platelets when in a low-oxygen environment. Endothelial growth and proliferation is also directly stimulated by hypoxia, and presence of lactic acid in the wound.

To migrate, endothelial cells need collagenases and plasminogen activator to degrade the clot and part of the ECM. Zinc-dependent metalloproteinases digest basement membrane and ECM to allow cell migration, proliferation and angiogenesis.

When macrophages and other growth factor-producing cells are no longer in a hypoxic, lactic acid-filled environment, they stop producing angiogenic factors. Thus, when tissue is adequately perfused, migration and proliferation of endothelial cells is reduced. Eventually blood vessels that are no longer needed die by apoptosis.

Fibroplasia and granulation tissue formation

Simultaneously with angiogenesis, fibroblasts begin accumulating in the wound site. Fibroblasts begin entering the wound site two to five days after wounding as the inflammatory phase is ending, and their numbers peak at one to two weeks post-wounding. By the end of the first week, fibroblasts are the main cells in the wound Fibroplasia ends two to four weeks after wounding.

In the first two or three days after injury, fibroblasts mainly migrate and proliferate, while later, they are the main cells that lay down the collagen matrix in the wound site. Origins of these fibroblasts are thought to be from the adjacent uninjured cutaneous tissue (although new evidence suggests that some are derived from blood-borne, circulating adult stem cells/precursors). Initially fibroblasts utilize the fibrin cross-linking fibers (well-formed by the end of the inflammatory phase) to migrate across the wound, subsequently adhering to fibronectin. Fibroblasts then deposit ground substance into the wound bed, and later collagen, which they can adhere to for migration.

Granulation tissue functions as rudimentary tissue, and begins to appear in the wound already during the inflammatory phase, two to five days post wounding, and continues growing until the wound bed is covered. Granulation tissue consists of new blood vessels, fibroblasts, inflammatory cells, endothelial cells, myofibroblasts, and the components of a new, provisional extracellular matrix (ECM). The provisional ECM is different in composition from the ECM in normal tissue and its components originate from fibroblasts. Such components include fibronectin, collagen, glycosaminoglycans, elastin, glycoproteins and proteoglycans. Its main components are fibronectin and hyaluronan, which create a very hydrated matrix and facilitate cell migration. Later this provisional matrix is replaced with an ECM that more closely resembles that found in non-injured tissue.

Growth factors (PDGF, TGF-β) and fibronectin encourage proliferation, migration to the wound bed, and production of ECM molecules by fibroblasts. Fibroblasts also secrete growth factors that attract epithelial cells to the wound site. Hypoxia also contributes to

fibroblast proliferation and excretion of growth factors, though too little oxygen will inhibit their growth and deposition of ECM components, and can lead to excessive, fibrotic scarring.

Collagen deposition

One of fibroblasts' most important duties is the production of collagen.

Collagen deposition is important because it increases the strength of the wound; before it is laid down, the only thing holding the wound closed is the fibrin-fibronectin clot, which does not provide much resistance to traumatic injury. Also, cells involved in inflammation, angiogenesis, and connective tissue construction attach to, grow and differentiate on the collagen matrix laid down by fibroblasts.

Type III collagen and fibronectin are generally beginning to be produced in appreciable amounts at somewhere between approximately 10 hours and 3 days, depending mainly on wound size. Their deposition peaks at one to three weeks. They are the predominating tensile substances until the later phase of maturation, in which they are replaced by the stronger type I collagen.

Even as fibroblasts are producing new collagen, collagenases and other factors degrade it. Shortly after wounding, synthesis exceeds degradation so collagen levels in the wound rise, but later production and degradation become equal so there is no net collagen gain. This homeostasis signals the onset of the later maturation phase. Granulation gradually ceases and fibroblasts decrease in number in the wound once their work is done. At the end of the granulation phase, fibroblasts begin to commit apoptosis, converting granulation tissue from an environment rich in cells to one that consists mainly of collagen.

Epithelialization

The formation of granulation tissue in an open wound allows the reepithelialization phase to take place, as epithelial cells migrate across the new tissue to form a barrier between the wound and the environment. Basal keratinocytes from the wound edges and dermal appendages such as hair follicles, sweat glands and sebacious (oil) glands are the main cells responsible for the epithelialization phase of wound healing. They advance in a sheet across the wound site and proliferate at its edges, ceasing movement when they meet in the middle.

Keratinocytes migrate without first proliferating. Migration can begin as early as a few hours after wounding. However, epithelial cells require viable tissue to migrate across, so if the wound is deep it must first be filled with granulation tissue. Thus the time of onset of migration is variable and may occur about one day after wounding. Cells on the wound margins proliferate on the second and third day post-wounding in order to provide more cells for migration.

If the basement membrane is not breached, epithelial cells are replaced within three days by division and upward migration of cells in the stratum basale in the same fashion that occurs in uninjured skin. However, if the basement membrane is ruined at the wound site, reepithelization must occur from the wound margins and from skin appendages such as hair follicles and sweat and oil glands that enter the dermis that are lined with viable keratinocytes. If the wound is very deep, skin appendages may also be ruined and migration can only occur from wound edges.

Migration of keratinocytes over the wound site is stimulated by lack of contact inhibition and by chemicals such as nitric oxide. Before they begin to migrate, cells must dissolve their desmosomes and hemidesmosomes, which normally anchor the cells by intermediate filaments in their cytoskeleton to other cells and to the ECM. Transmembrane receptor proteins called integrins, which are made of glycoproteins and normally anchor the cell to the basement membrane by its cytoskeleton, are released from the cell's intermediate filaments and relocate to actin filaments to serve as attachments to the ECM for pseudopodia during migration. Thus keratinocytes detach from the basement membrane and are able to enter the wound bed.

Before they begin migrating, keratinocytes change shape, becoming longer and flatter and extending cellular processes like lamellipodia and wide processes that look like ruffles. Actin filaments and pseudopodia form. During migration, integrins on the pseudopod attach to the ECM, and the actin filaments in the projection pull the cell along. The interaction with molecules in the ECM through integrins further promotes the formation of actin filaments, lamellipodia, and filopodia.

Epithelial cells climb over one another in order to migrate. This growing sheet of epithelial cells is often called the epithelial tongue. The first cells to attach to the basement membrane form the stratum basale. These basal cells continue to migrate across the wound bed, and epithelial cells above them slide along as well. The more quickly this migration occurs, the less of a scar there will be.

Fibrin, collagen, and fibronectin in the ECM may further signal cells to divide and migrate. Like fibroblasts, migrating keratinocytes use the fibronectin cross-linked with fibrin that was deposited in inflammation as an attachment site to crawl across.

As keratinocytes migrate, they move over granulation tissue but underneath the scab (if one was formed), separating it from the underlying tissue. Epithelial cells have the ability to phagocytize debris such as dead tissue and bacterial matter that would otherwise obstruct their path. Because they must dissolve any scab that forms, keratinocyte migration is best enhanced by a moist environment, since a dry one leads to formation of a bigger, tougher scab. To make their way along the tissue, keratinocytes must dissolve the clot, debris, and parts of the ECM in order to get through. They secrete plasminogen activator, which activates plasminogen, turning it into plasmin to dissolve the scab. Cells can only migrate over living tissue, so they must excrete collagenases and proteases like matrix metalloproteinases (MMPs) to dissolve damaged parts of the ECM in their way,

particularly at the front of the migrating sheet. Keratinocytes also dissolve the basement membrane, using instead the new ECM laid down by fibroblasts to crawl across.

As keratinocytes continue migrating, new epithelial cells must be formed at the wound edges to replace them and to provide more cells for the advancing sheet. Proliferation behind migrating keratinocytes normally begins a few days after wounding and occurs at a rate that is 17 times higher in this stage of epithelialization than in normal tissues. Until the entire wound area is resurfaced, the only epithelial cells to proliferate are at the wound edges.

Growth factors, stimulated by integrins and MMPs, cause cells to proliferate at the wound edges. Keratinocytes themselves also produce and secrete factors, including growth factors and basement membrane proteins, which aid both in epithelialization and in other phases of healing. Growth factors are also important for the innate immune defense of skin wounds by stimulation of the production of antimicrobial peptides and neutrophil chemotactic cytokines in keratinocytes.

Keratinocytes continue migrating across the wound bed until cells from either side meet in the middle, at which point contact inhibition causes them to stop migrating. When they have finished migrating, the keratinocytes secrete the proteins that form the new basement membrane. Cells reverse the morphological changes they underwent in order to begin migrating; they reestablish desmosomes and hemidesmosomes and become anchored once again to the basement membrane. Basal cells begin to divide and differentiate in the same manner as they do in normal skin to reestablish the strata found in reepithelialized skin.

Contraction

Contraction is a key phase of wound healing. If contraction continues for too long, it can lead to disfigurement and loss of function. Thus there is a great interest in understanding the biology of wound contraction, which can be modelled in vitro using the collagen gel contraction assay or the dermal equivalent model.

Contraction commences approximately a week after wounding, when fibroblasts have differentiated into myofibroblasts In full thickness wounds, contraction peaks at 5 to 15 days post wounding. Contraction can last for several weeks and continues even after the wound is completely reepithelialized. A large wound can become 40 to 80% smaller after contraction. Wounds can contract at a speed of up to 0.75 mm per day, depending on how loose the tissue in the wounded area is. Contraction usually does not occur symmetrically; rather most wounds have an 'axis of contraction' which allows for greater organization and alignment of cells with collagen.

At first, contraction occurs without myofibroblast involvement. Later, fibroblasts, stimulated by growth factors, differentiate into myofibroblasts. Myofibroblasts, which are similar to smooth muscle cells, are responsible for contraction. Myofibroblasts contain the same kind of actin as that found in smooth muscle cells.

Myofibroblasts are attracted by fibronectin and growth factors and they move along fibronectin linked to fibrin in the provisional ECM in order to reach the wound edges. They form connections to the ECM at the wound edges, and they attach to each other and to the wound edges by desmosomes. Also, at an adhesion called the fibronexus, actin in the myofibroblast is linked across the cell membrane to molecules in the extracellular matrix like fibronectin and collagen. Myofibroblasts have many such adhesions, which allow them to pull the ECM when they contract, reducing the wound size. In this part of contraction, closure occurs more quickly than in the first, myofibroblast-independent part.

As the actin in myofibroblasts contracts, the wound edges are pulled together. Fibroblasts lay down collagen to reinforce the wound as myofibroblasts contract The contraction stage in proliferation ends as myofibroblasts stop contracting and commit apoptosis. The breakdown of the provisional matrix leads to a decrease in hyaluronic acid and an increase in chondroitin sulfate, which gradually triggers fibroblasts to stop migrating and proliferating. These events signal the onset of the maturation stage of wound healing.

Maturation and remodeling

When the levels of collagen production and degradation equalize, the maturation phase of tissue repair is said to have begun. During maturation, type III collagen, which is prevalent during proliferation, is gradually degraded and the stronger type I collagen is laid down in its place. Originally disorganized collagen fibers are rearranged, cross-linked, and aligned along tension lines. The onset of the maturation phase may vary extensively, depending on the size of the wound and whether it was initially closed or left open, ranging from approximately 3 days to 3 weeks The maturation phase can last for a year or longer, similarly depending on wound type.

As the phase progresses, the tensile strength of the wound increases, with the strength approaching 50% that of normal tissue by three months after injury and ultimately becoming as much as 80% as strong as normal tissue. Since activity at the wound site is reduced, the scar loses its red appearance as blood vessels that are no longer needed are removed by apoptosis.

The phases of wound healing normally progress in a predictable, timely manner; if they do not, healing may progress inappropriately to either a chronic wound such as a venous ulcer or pathological scarring such as a keloid scar.

Research and development

Up until a decade ago, the classic paradigm of wound healing, involving stem cells restricted to organ-specific lineages, has never been seriously challenged. Since then, the notion of adult stem cells having cellular *plasticity* or the ability to differentiate into non-lineage cells has emerged as an alternative explanation. To be more specific, hematopoietic progenitor cells (that give rise mature cells in the blood) may have the

ability *de-differentiate* back into hematopoietic stem cells and/or *transdifferentiate* into non-lineage cells, such as fibroblasts.

Stem cells and cellular plasticity

Multipotent adult stem cells have the capacity to be self-renewal and give rise to different cell types. Stem cells give rise to progenitor cells, which are cells that are not self-renewal, but can generate several types of cells. The extent of stem cell involvement in cutaneous (skin) wound healing is complex and not fully understood.

It is thought that the epidermis and dermis are reconstituted by mitotically active stem cells that reside at the apex of rete ridges (basal stem cells or BSC), the bulge of hair follicles (hair follicular stem cell or HFSC), and the papillary dermis (dermal stem cells). Moreover, bone marrow may also contain stem cells that play a major role in cutaneous wound healing.

In rare circumstances, such as extensive cutaneous injury, self-renewal subpopulations in the bone marrow are induced to participate in the healing process, whereby they give rise to collagen-secreting cells that seem to play a role during wound repair. These two self-renewal subpopulations are (1) bone marrow-derived mesenchymal stem cells (MSC) and (2) hematopoietic stem cells (HSC). Bone marrow also harbors a progenitor subpopulation (endothelial progenitor cells or EPC) that, in the same type of setting, are mobilized to aid in the reconstruction of blood vessels. Moreover, it thought that, extensive injury to skin also promotes the early trafficking of a unique subclass of leukocytes (circulating fibrocytes) to the injured region, where they perform various functions related to wound healing.

Wound repair versus regeneration

There is a subtle distinction between ‘repair’ and ‘regeneration'. An injury is an interruption of morphology and/or functionality of a given tissue. Repair refers to the physiologic adaptation of an organ after injury in an effort to re-establish continuity without regards to exact replacement of lost/damaged tissue. True tissue regeneration refers to the replacement of lost/damaged tissue with an ‘exact’ copy, such that both morphology and functionality are completely restored. Mammals do not regenerate spontaneously. In some instances, such as skin, ‘partial regeneration’ may be induced by the use of biodegradable (collagen-glycoaminoglycan) scaffolds. These scaffolds are structurally analogous to extracellular matrix (ECM) found in normal/un-injured dermis. Interestingly, fundamental conditions required for tissue regeneration often oppose conditions that favor efficient wound repair, including inhibition of (1) platelet activation, (2) inflammatory response, and (3) wound contraction. In addition to providing support for fibroblast and endothelial cell attachment, biodegradable scaffolds inhibit wound contraction, thereby allowing the healing process to proceed towards a more-regenerative/less-scarring pathway.

Types

Primary intention

involves epidermis and dermis without total penetration of dermis healing by process of epithelialization

- When wound edges are brought together so that they are adjacent to each other (re-approximated)
- Minimizes scarring
- Most surgical wounds heal by primary intention healing
- Wound closure is performed with sutures (stitches), staples, or adhesive tape
- Examples: well-repaired lacerations,well reduced bone fractures,healing after flap surgery

Secondary intention

- The wound is allowed to granulate
- Surgeon may pack the wound with a gauze or use a drainage system
- Granulation results in a broader scar
- Healing process can be slow due to presence of drainage from infection
- Wound care must be performed daily to encourage wound debris removal to allow for granulation tissue formation
- examples:gingivectomy,gingivoplasty,tooth extraction sockets, poorly reduced fractures.

Tertiary intention

(Delayed primary closure or secondary suture):

- The wound is initially cleaned, debrided and observed, typically 4 or 5 days before closure.
- The wound is purposely left open
- examples:healing of wounds by use of tissue grafts.

Overview of involved growth factors

Following are the main growth factors involved in wound healing:

Growth factor	Abbreviation	Main origins	Effects
Epidermal growth factor	EGF	• Activated macrophages • Salivary glands • Keratinocytes	• Keratinocyte and fibroblast mitogen • Keratinocyte migration • Granulation tissue

			formation
Transforming growth factor-α	TGF-α	• Activated macrophages • T-lymphocytes • Keratinocytes	• Hepatocyte and epithelial cell proliferation • Expression of antimicrobial peptides • Expression of chemotactic cytokines
Hepatocyte growth factor	HGF	• Mesenchymal cells	• Epithelial and endothelial cell proliferation • Hepatocyte motility
Vascular endothelial growth factor	VEGF	• Mesenchymal cells	• Vascular permeability • Endothelial cell proliferation
Platelet derived growth factor	PDGF	• Platelets • Macrophages • Endothelial cells • Smooth muscle cells • Keratinocytes	• Granulocyte, macrophage, fibroblast and smooth muscle cell chemotaxis • Granulocyte, macrophage and fibroblast activation • Fibroblast, endothelial cell and smooth muscle cell proliferation • Matrix metalloproteinase, fibronectin and hyaluronan production • Angiogenesis • Wound remodeling • Integrin expression

			regulation
Fibroblast growth factor 1 and 2	FGF-1, -2	• Macrophages • Mast cells • T-lymphocytes • Endothelial cells • Fibroblasts	• Fibroblast chemotaxis • Fibroblast and keratinocyte proliferation • Keratinocyte migration • Angiogenesis • Wound contraction • matrix deposition
Transforming growth factor-β	TGF-β	• Platelets • T-lymphocytes • Macrophages • Endothelial cells • Keratinocytes • Smooth muscle cells • Fibroblasts	• Granulocyte, macrophage, lymphocyte, fibroblast and smooth muscle cell chemotaxis • TIMP synthesis • Angiogenesis • Fibroplasia • Matrix metalloproteinase production inhibition • Keratinocyte proliferation
Keratinocyte growth factor	KGF	• Keratinocytes	• Keratinocyte migration, proliferation and differentiation

Chapter 2

Coagulation

Coagulation is a complex process by which blood forms clots. It is an important part of hemostasis (the cessation of blood loss from a damaged vessel), wherein a damaged blood vessel wall is covered by a platelet and fibrin-containing clot to stop bleeding and begin repair of the damaged vessel. Disorders of coagulation can lead to an increased risk of bleeding (hemorrhage) or obstructive clotting (thrombosis).

Coagulation is highly conserved throughout biology; in all mammals, coagulation involves both a cellular (platelet) and a protein (coagulation factor) component. The system in humans has been the most extensively researched and is therefore the best understood.

Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium (lining of the vessel). Exposure of the blood to proteins such as tissue factor initiates changes to blood platelets and the plasma protein fibrinogen, a clotting factor. Platelets immediately form a plug at the site of injury; this is called *primary hemostasis*. *Secondary hemostasis* occurs simultaneously: Proteins in the blood plasma, called *coagulation factors* or *clotting factors*, respond in a complex cascade to form fibrin strands, which strengthen the platelet plug.

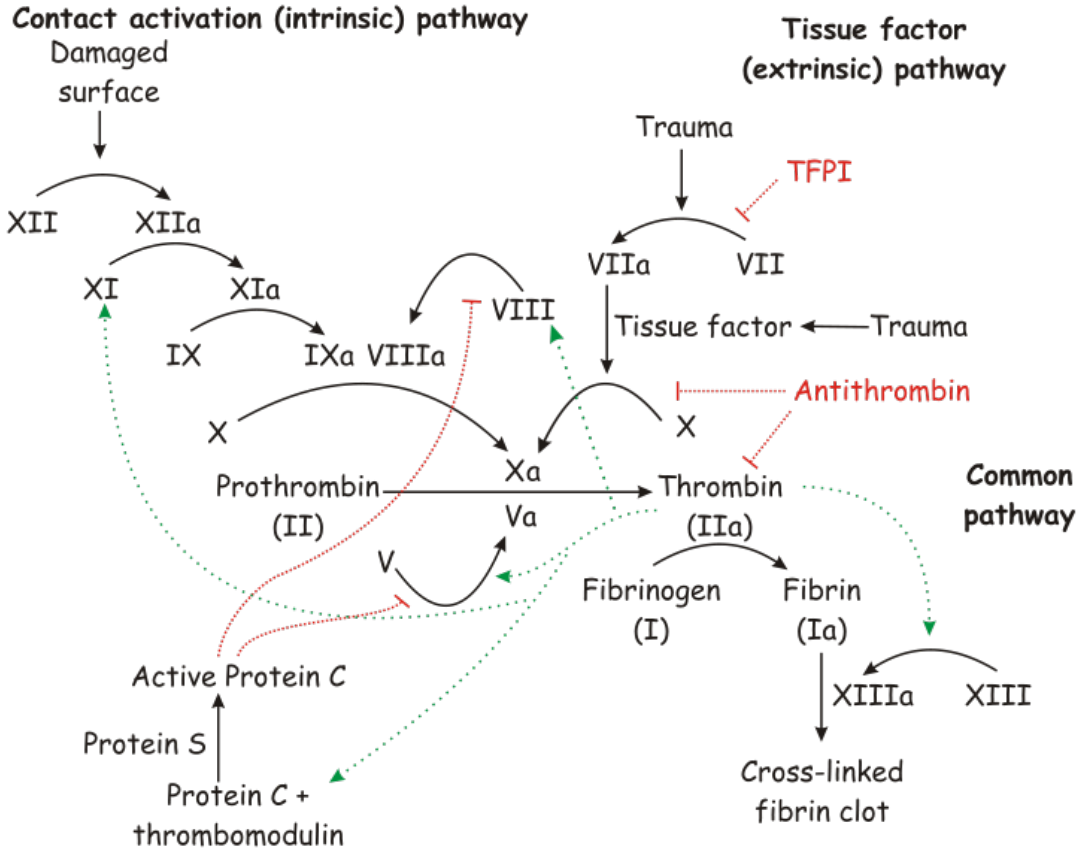
Physiology

Platelet activation

Damage to blood vessel walls exposes subendothelium proteins, most notably von Willebrand factor (vWF), present under the endothelium. vWF is a protein secreted by healthy endothelium, forming a layer between the endothelium and underlying basement membrane. When the endothelium is damaged, the normally-isolated, underlying vWF is exposed to white blood cells and recruits Factor VIII, collagen, and other clotting factors. Circulating platelets bind to collagen with surface collagen-specific glycoprotein Ia/IIa receptors. This adhesion is strengthened further by additional circulating proteins vWF, which forms additional links between the platelets glycoprotein Ib/IX/V and the collagen fibrils. These adhesions activate the platelets.

Activated platelets release the contents of stored granules into the blood plasma. The granules include ADP, serotonin, platelet-activating factor (PAF), vWF, platelet factor 4, and thromboxane A_2 (TXA_2), which, in turn, activate additional platelets. The granules' contents activate a G_q-linked protein receptor cascade, resulting in increased calcium concentration in the platelets' cytosol. The calcium activates protein kinase C, which, in turn, activates phospholipase A_2 (PLA_2). PLA_2 then modifies the integrin membrane glycoprotein IIb/IIIa, increasing its affinity to bind fibrinogen. The activated platelets change shape from spherical to stellate, and the fibrinogen cross-links with glycoprotein IIb/IIIa aid in aggregation of adjacent platelets (completing primary hemostasis).

The coagulation cascade



The coagulation cascade of secondary hemostasis.

The coagulation cascade of secondary hemostasis has two pathways which lead to *fibrin* formation. These are the *contact activation pathway* (formerly known as the intrinsic pathway), and the *tissue factor pathway* (formerly known as the extrinsic pathway). It was previously thought that the coagulation cascade consisted of two pathways of equal importance joined to a common pathway. It is now known that the primary pathway for the initiation of blood coagulation is the *tissue factor* pathway. The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the

next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase *a* appended to indicate an active form.

The coagulation factors are generally serine proteases (enzymes). There are some exceptions. For example, FVIII and FV are glycoproteins, and Factor XIII is a transglutaminase. Serine proteases act by cleaving other proteins at specific sites. The coagulation factors circulate as inactive zymogens. The coagulation cascade is classically divided into three pathways. The *tissue factor* and *contact activation* pathways both activate the "final common pathway" of factor X, thrombin and fibrin.

Tissue factor pathway (extrinsic)

The main role of the tissue factor pathway is to generate a "thrombin burst," a process by which thrombin, the most important constituent of the coagulation cascade in terms of its feedback activation roles, is released instantaneously. FVIIa circulates in a higher amount than any other activated coagulation factor.

- Following damage to the blood vessel, FVII leaves the circulation and comes into contact with tissue factor (TF) expressed on tissue-factor-bearing cells (stromal fibroblasts and leukocytes), forming an activated complex (TF-FVIIa).
- TF-FVIIa activates FIX and FX.
- FVII is itself activated by thrombin, FXIa, FXII and FXa.
- The activation of FXa by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI).
- FXa and its co-factor FVa form the prothrombinase complex, which activates prothrombin to thrombin.
- Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which activates FXI, which, in turn, activates FIX), and activates and releases FVIII from being bound to vWF.
- FVIIIa is the co-factor of FIXa, and together they form the "tenase" complex, which activates FX; and so the cycle continues. ("Tenase" is a contraction of "ten" and the suffix "-ase" used for enzymes.)

Contact activation pathway (intrinsic)

The contact activation pathway begins with formation of the primary complex on collagen by high-molecular-weight kininogen (HMWK), prekallikrein, and FXII (Hageman factor). Prekallikrein is converted to kallikrein and FXII becomes FXIIa. FXIIa converts FXI into FXIa. Factor XIa activates FIX, which with its co-factor FVIIIa form the tenase complex, which activates FX to FXa. The minor role that the contact activation pathway has in initiating clot formation can be illustrated by the fact that patients with severe deficiencies of FXII, HMWK, and prekallikrein do not have a bleeding disorder. Instead, contact activation system seems to be more involved in inflammation. Patients without FXII (Hageman factor) suffer from constant infections.

Final common pathway

Thrombin has a large array of functions. Its primary role is the conversion of fibrinogen to fibrin, the building block of a hemostatic plug. In addition, it activates Factors VIII and V and their inhibitor protein C (in the presence of thrombomodulin), and it activates Factor XIII, which forms covalent bonds that crosslink the fibrin polymers that form from activated monomers.

Following activation by the contact factor or tissue factor pathways, the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex, until it is down-regulated by the anticoagulant pathways.

Cofactors

Various substances are required for the proper functioning of the coagulation cascade:

- Calcium and phospholipid (a platelet membrane constituent) are required for the tenase and prothrombinase complexes to function. Calcium mediates the binding of the complexes via the terminal gamma-carboxy residues on FXa and FIXa to the phospholipid surfaces expressed by platelets, as well as procoagulant microparticles or microvesicles shed from them. Calcium is also required at other points in the coagulation cascade.
- Vitamin K is an essential factor to a hepatic gamma-glutamyl carboxylase that adds a carboxyl group to glutamic acid residues on factors II, VII, IX and X, as well as Protein S, Protein C and Protein Z. In adding the gamma-carboxyl group to glutamate residues on the immature clotting factors Vitamin K is itself oxidized. Another enzyme, *Vitamin K epoxide reductase*, (VKORC) reduces vitamin K back to its active form. Vitamin K epoxide reductase is pharmacologically important as a target for anticoagulant drugs warfarin and related coumarins such as acenocoumarol, phenprocoumon, and dicumarol. These drugs create a deficiency of reduced vitamin K by blocking VKORC, thereby inhibiting maturation of clotting factors. Other deficiencies of vitamin K (e.g., in malabsorption), or disease (hepatocellular carcinoma) impairs the function of the enzyme and leads to the formation of PIVKAs (proteins formed in vitamin K absence); this causes partial or non-gamma carboxylation, and affects the coagulation factors' ability to bind to expressed phospholipid.

Regulators

Five mechanisms keep platelet activation and the coagulation cascade in check. Abnormalities can lead to an increased tendency toward thrombosis:

- Protein C is a major physiological anticoagulant. It is a vitamin K-dependent serine protease enzyme that is activated by thrombin into activated protein C (APC). Protein C is activated in a sequence that starts with Protein C and thrombin binding to a cell surface protein thrombomodulin. Thrombomodulin

binds these proteins in such a way that it activates Protein C. The activated form, along with protein S and a phospholipid as cofactors, degrades FVa and FVIIIa. Quantitative or qualitative deficiency of either may lead to thrombophilia (a tendency to develop thrombosis). Impaired action of Protein C (activated Protein C resistance), for example by having the "Leiden" variant of Factor V or high levels of FVIII also may lead to a thrombotic tendency.

- Antithrombin is a serine protease inhibitor (serpin) that degrades the serine proteases: thrombin, FIXa, FXa, FXIa, and FXIIa. It is constantly active, but its adhesion to these factors is increased by the presence of heparan sulfate (a glycosaminoglycan) or the administration of heparins (different heparinoids increase affinity to FXa, thrombin, or both). Quantitative or qualitative deficiency of antithrombin (inborn or acquired, e.g., in proteinuria) leads to thrombophilia.
- Tissue factor pathway inhibitor (TFPI) limits the action of tissue factor (TF). It also inhibits excessive TF-mediated activation of FIX and FX.
- Plasmin is generated by proteolytic cleavage of plasminogen, a plasma protein synthesized in the liver. This cleavage is catalyzed by tissue plasminogen activator (t-PA), which is synthesized and secreted by endothelium. Plasmin proteolytically cleaves fibrin into fibrin degradation products that inhibit excessive fibrin formation.
- Prostacyclin (PGI_2) is released by endothelium and activates platelet G_s protein-linked receptors. This, in turn, activates adenylyl cyclase, which synthesizes cAMP. cAMP inhibits platelet activation by decreasing cytosolic levels of calcium and, by doing so, inhibits the release of granules that would lead to activation of additional platelets and the coagulation cascade.

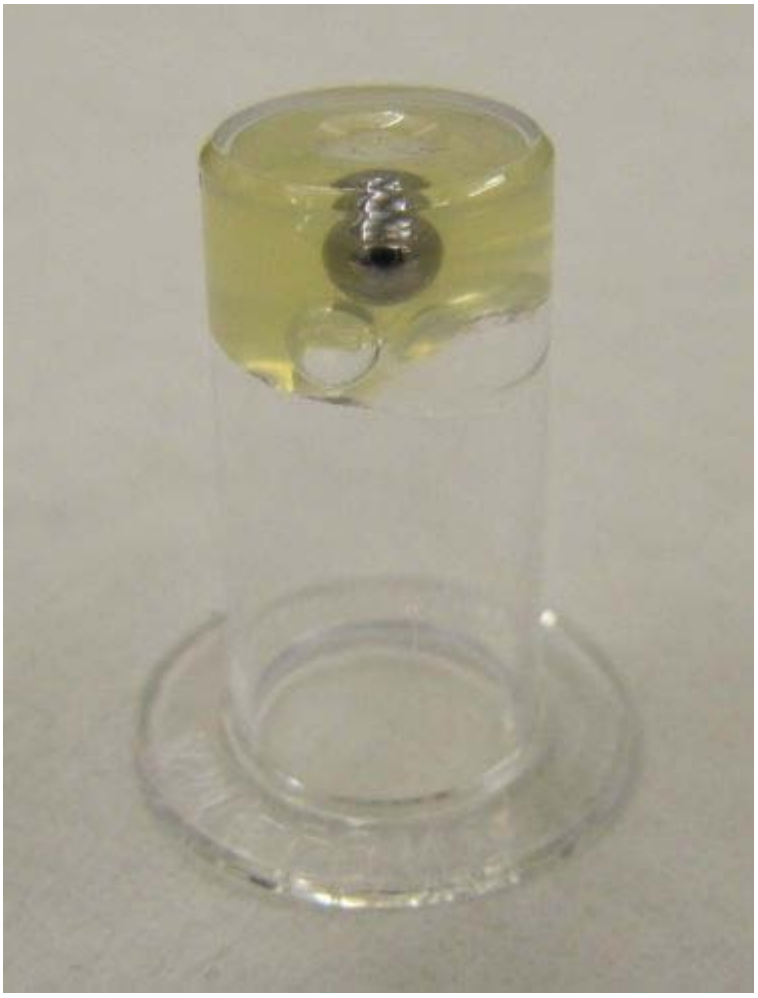
Fibrinolysis

Eventually, blood clots are reorganised and resorbed by a process termed *fibrinolysis*. The main enzyme responsible for this process (plasmin) is regulated by various activators and inhibitors.

Role in immune system

The coagulation system overlaps with the immune system. Coagulation can physically trap invading microbes in blood clots. Also, some products of the coagulation system can contribute to the innate immune system by their ability to increase vascular permeability and act as chemotactic agents for phagocytic cells. In addition, some of the products of the coagulation system are directly antimicrobial. For example, beta-lysine, a protein produced by platelets during coagulation, can cause lysis of many Gram-positive bacteria by acting as a cationic detergent. Many acute-phase proteins of inflammation are involved in the coagulation system. In addition, pathogenic bacteria may secrete agents that alter the coagulation system, e.g. coagulase and streptokinase.

Testing of coagulation



Blood plasma after the addition of Tissue Factor forms a gel-like structure (Test for prothrombin time).

Numerous tests are used to assess the function of the coagulation system:

- Common: aPTT, PT (also used to determine INR), fibrinogen testing (often by the Clauss method), platelet count, platelet function testing (often by PFA-100).
- Other: TCT, bleeding time, mixing test (whether an abnormality corrects if the patient's plasma is mixed with normal plasma), coagulation factor assays, antiphosholipid antibodies, D-dimer, genetic tests (e.g. factor V Leiden, prothrombin mutation G20210A), dilute Russell's viper venom time (dRVVT), miscellaneous platelet function tests, thromboelastography (TEG or Sonoclot), euglobulin lysis time (ELT), .

The contact activation (intrinsic) pathway is initiated by activation of the "contact factors" of plasma, and can be measured by the activated partial thromboplastin time (aPTT) test.

The tissue factor (extrinsic) pathway is initiated by release of tissue factor (a specific cellular lipoprotein), and can be measured by the prothrombin time (PT) test. PT results are often reported as ratio (INR value) to monitor dosing of oral anticoagulants such as warfarin.

The quantitative and qualitative screening of fibrinogen is measured by the thrombin clotting time (TCT). Measurement of the exact amount of fibrinogen present in the blood is generally done using the Clauss method for fibrinogen testing. Many analysers are capable of measuring a "derived fibrinogen" level from the graph of the Prothrombin time clot.

If a coagulation factor is part of the contact activation or tissue factor pathway, a deficiency of that factor will affect only one of the tests: Thus hemophilia A, a deficiency of factor VIII, which is part of the contact activation pathway, results in an abnormally prolonged aPTT test but a normal PT test. The exceptions are prothrombin, fibrinogen, and some variants of FX that can be detected only by either aPTT or PT. If an abnormal PT or aPTT is present, additional testing will occur to determine which (if any) factor is present as aberrant concentrations.

Deficiencies of fibrinogen (quantitative or qualitative) will affect all screening tests.

Condition	Prothrombin time	Partial thromboplastin time	Bleeding time	Platelet count
Vitamin K deficiency or warfarin	prolonged	prolonged	unaffected	unaffected
Disseminated intravascular coagulation	prolonged	prolonged	prolonged	decreased
Von Willebrand disease	unaffected	prolonged	prolonged	unaffected
Haemophilia	unaffected	prolonged	unaffected	unaffected
Aspirin	unaffected	unaffected	prolonged	unaffected
Thrombocytopenia	unaffected	unaffected	prolonged	decreased
Early Liver failure	prolonged	unaffected	unaffected	unaffected
End-stage Liver failure	prolonged	prolonged	prolonged	decreased
Uremia	unaffected	unaffected	prolonged	unaffected
Congenital afibrinogenemia	prolonged	prolonged	prolonged	unaffected
Factor V deficiency	prolonged	prolonged	unaffected	unaffected
Factor X deficiency as seen in amyloid purpura	prolonged	prolonged	unaffected	unaffected
Glanzmann's thrombasthenia	unaffected	unaffected	prolonged	unaffected

Bernard-Soulier syndrome	unaffected	unaffected	prolonged	decreased

Role in disease

Problems with coagulation may dispose to hemorrhage, thrombosis, and occasionally both, depending on the nature of the pathology.

Platelet disorders

Platelet conditions may be inborn or acquired. Some inborn platelet pathologies are Glanzmann's thrombasthenia, Bernard-Soulier syndrome (abnormal glycoprotein Ib-IX-V complex), gray platelet syndrome (deficient alpha granules), and delta storage pool deficiency (deficient dense granules). Most are rare conditions. Most inborn platelet pathologies predispose to hemorrhage. Von Willebrand disease is due to deficiency or abnormal function of von Willebrand factor, and leads to a similar bleeding pattern; its milder forms are relatively common.

Decreased platelet numbers may be due to various causes, including insufficient production (e.g., in myelodysplastic syndrome or other bone marrow disorders), destruction by the immune system (immune thrombocytopenic purpura/ITP), and consumption due to various causes (thrombotic thrombocytopenic purpura/TTP, hemolytic-uremic syndrome/HUS, paroxysmal nocturnal hemoglobinuria/PNH, disseminated intravascular coagulation/DIC, heparin-induced thrombocytopenia/HIT). Most consumptive conditions lead to platelet activation, and some are associated with thrombosis.

Disease and clinical significance of thrombosis

The best-known coagulation factor disorders are the hemophilias. The three main forms are hemophilia A (factor VIII deficiency), hemophilia B (factor IX deficiency or "Christmas disease") and hemophilia C (factor XI deficiency, mild bleeding tendency). Hemophilia A and B are X-linked recessive disorders, whereas Hemophilia C is much more rare autosomal recessive disorder most commonly seen in Ashkenazi Jews.

Von Willebrand disease (which behaves more like a platelet disorder except in severe cases), is the most common hereditary bleeding disorder and is characterized as being inherited autosomal recessive or dominant. In this disease, there is a defect in von Willebrand factor (vWF), which mediates the binding of glycoprotein Ib (GPIb) to collagen. This binding helps mediate the activation of platelets and formation of primary hemostasis.

Bernard-Soulier syndrome is a defect or deficiency in GPIb. GPIb, the receptor for vWF, can be defective and lead to lack of primary clot formation (primary hemostasis) and increased bleeding tendency. This is an autosomal recessive inherited disorder.

Thrombasthenia of Glanzman and Naegeli (Glanzmann thrombasthenia) is extremely rare. It is characterized by a defect in GPIIb/IIIa fibrinogen receptor complex. When GPIIb/IIIa receptor is dysfunctional, fibrinogen cannot cross-link platelets, which inhibits primary hemostasis. This is an autosomal recessive inherited disorder.

In liver failure (acute and chronic forms), there is insufficient production of coagulation factors by the liver; this may increase bleeding risk.

Deficiency of Vitamin K may also contribute to bleeding disorders because clotting factor maturation depends on Vitamin K.

Thrombosis is the pathological development of blood clots. These clots may break free and become mobile, forming an embolus or grow to such a size that occludes the vessel in which it developed. An embolism is said to occur when the thrombus (blood clot) becomes a mobile embolus and migrates to another part of the body, interfering with blood circulation and hence impairing organ function downstream of the occlusion. This causes ischemia and often leads to ischemic necrosis of tissue. Most cases of thrombosis are due to acquired extrinsic problems (surgery, cancer, immobility, obesity, economy class syndrome), but a small proportion of people harbor predisposing conditions known collectively as thrombophilia (e.g., antiphospholipid syndrome, factor V Leiden, and various other rarer genetic disorders).

Mutations in factor XII have been associated with an asymptomatic prolongation in the clotting time and possibly a tendency toward thrombophlebitis. Other mutations have been linked with a rare form of hereditary angioedema (type III).

Pharmacology

Procoagulants

The use of adsorbent chemicals, such as zeolites, and other hemostatic agents are also used for use in sealing severe injuries quickly (such as in traumatic bleeding secondary to gunshot wounds). Thrombin and fibrin glue are used surgically to treat bleeding and to thrombose aneurysms.

Desmopressin is used to improve platelet function by activating arginine vasopressin receptor 1A.

Coagulation factor concentrates are used to treat hemophilia, to reverse the effects of anticoagulants, and to treat bleeding in patients with impaired coagulation factor synthesis or increased consumption. Prothrombin complex concentrate, cryoprecipitate and fresh frozen plasma are commonly-used coagulation factor products. Recombinant activated human factor VII is increasingly popular in the treatment of major bleeding.

Tranexamic acid and aminocaproic acid inhibit fibrinolysis, and lead to a *de facto* reduced bleeding rate. Before its withdrawal, aprotinin was used in some forms of major surgery to decrease bleeding risk and need for blood products.

Anticoagulants

Anticoagulants and anti-platelet agents are amongst the most commonly used medications. Anti-platelet agents include aspirin, clopidogrel, dipyridamole and ticlopidine; the parenteral glycoprotein IIb/IIIa inhibitors are used during angioplasty. Of the anticoagulants, warfarin (and related coumarins) and heparin are the most commonly used. Warfarin affects the vitamin K-dependent clotting factors (II, VII, IX,X) , whereas heparin and related compounds increase the action of antithrombin on thrombin and factor Xa. A newer class of drugs, the direct thrombin inhibitors, is under development; some members are already in clinical use (such as lepirudin). Also under development are other small molecular compounds that interfere directly with the enzymatic action of particular coagulation factors (e.g., rivaroxaban, dabigatran, apixaban).

Coagulation factors

Coagulation factors and related substances

Number and/or name	Function
I (fibrinogen)	Forms clot (fibrin)
II (prothrombin)	Its active form (IIa) activates I, V, VII, VIII, XI, XIII, protein C, platelets
Tissue factor	Co-factor of VIIa (formerly known as factor III)
Calcium	Required for coagulation factors to bind to phospholipid (formerly known as factor IV)
V (proaccelerin, labile factor)	Co-factor of X with which it forms the prothrombinase complex
VI	*Unassigned* – old name of Factor Va
VII (stable factor, proconvertin)	Activates IX, X
VIII (Antihemophilic factor A)	Co-factor of IX with which it forms the tenase complex
IX (Antihemophilic factor B or Christmas factor)	Activates X: forms tenase complex with factor VIII
X (Stuart-Prower factor)	Activates II: forms prothrombinase complex with factor V
XI (plasma thromboplastin antecedent)	Activates IX
XII (Hageman factor)	Activates factor XI, VII and prekallikrein
XIII (fibrin-stabilizing factor)	Crosslinks fibrin
von Willebrand factor	Binds to VIII, mediates platelet adhesion

prekallikrein (Fletcher factor)	Activates XII and prekallikrein; cleaves HMWK
high-molecular-weight kininogen (HMWK) (Fitzgerald factor)	Supports reciprocal activation of XII, XI, and prekallikrein
fibronectin	Mediates cell adhesion
antithrombin III	Inhibits IIa, Xa, and other proteases;
heparin cofactor II	Inhibits IIa, cofactor for heparin and dermatan sulfate ("minor antithrombin")
protein C	Inactivates Va and VIIIa
protein S	Cofactor for activated protein C (APC, inactive when bound to C4b-binding protein)
protein Z	Mediates thrombin adhesion to phospholipids and stimulates degradation of factor X by ZPI
Protein Z-related protease inhibitor (ZPI)	Degrades factors X (in presence of protein Z) and XI (independently)
plasminogen	Converts to plasmin, lyses fibrin and other proteins
alpha 2-antiplasmin	Inhibits plasmin
tissue plasminogen activator (tPA)	Activates plasminogen
urokinase	Activates plasminogen
plasminogen activator inhibitor-1 (PAI1)	Inactivates tPA & urokinase (endothelial PAI)
plasminogen activator inhibitor-2 (PAI2)	Inactivates tPA & urokinase (placental PAI)
cancer procoagulant	Pathological factor X activator linked to thrombosis in cancer

History

Initial discoveries

Theories on the coagulation of blood have existed since antiquity. Physiologist Johannes Müller (1801-1858) described fibrin, the substance of a thrombus. Its soluble precursor, fibrinogen, was thus named by Rudolf Virchow (1821-1902), and isolated chemically by Prosper Sylvain Denis (1799-1863). Alexander Schmidt suggested that the conversion from fibrinogen to fibrin is the result of an enzymatic process, and labeled the hypothetical enzyme "thrombin" and its precursor "prothrombin". Arthus discovered in 1890 that calcium was essential in coagulation. Platelets were identified in 1865, and their function was elucidated by Giulio Bizzozero in 1882.

The theory that thrombin is generated by the presence of tissue factor was consolidated by Paul Morawitz in 1905. At this stage, it was known that *thrombokinase/thromboplastin* (factor III) is released by damaged tissues, reacting with

prothrombin (II), which, together with calcium (IV), forms *thrombin*, which converts fibrinogen into *fibrin* (I).

Coagulation factors

The remainder of the biochemical factors in the process of coagulation were largely discovered in the 20th century.

A first clue as to the actual complexity of the system of coagulation was the discovery of *proaccelerin* (initially and later called Factor V) by Paul Owren (1905-1990) in 1947. He also postulated its function to be the generation of accelerin (Factor VI), which later turned out to be the activated form of V (or Va); hence, VI is not now in active use.

Factor VII (also known as *serum prothrombin conversion accelerator* or *proconvertin*, precipitated by barium sulfate) was discovered in a young female patient in 1949 and 1951 by different groups.

Factor VIII turned out to be deficient in the clinically recognised but etiologically elusive hemophilia A; it was identified in the 1950s and is alternatively called *antihemophilic globulin* due to its capability to correct hemophilia A.

Factor IX was discovered in 1952 in a young patient with hemophilia B named Stephen Christmas (1947-1993). His deficiency was described by Dr. Rosemary Biggs and Professor R.G. MacFarlane in Oxford, UK. The factor is, hence, called Christmas Factor. Christmas lived in Canada, and campaigned for blood transfusion safety until succumbing to transfusion-related AIDS at age 46. An alternative name for the factor is *plasma thromboplastin component*, given by an independent group in California.

Hageman factor, now known as factor XII, was identified in 1955 in an asymptomatic patient with a prolonged bleeding time named of John Hageman. Factor X, or Stuart-Prower factor, followed, in 1956. This protein was identified in a Ms. Audrey Prower of London, who had a lifelong bleeding tendency. In 1957, an American group identified the same factor in a Mr. Rufus Stuart. Factors XI and XIII were identified in 1953 and 1961, respectively.

The view that the coagulation process is a "cascade" or "waterfall" was enunciated almost simultaneously by MacFarlane in the UK and by Davie and Ratnoff in the USA, respectively.

Nomenclature

The usage of Roman numerals rather than eponyms or systematic names was agreed upon during annual conferences (starting in 1955) of hemostasis experts. In 1962, consensus was achieved on the numbering of factors I-XII. This committee evolved into the present-day International Committee on Thrombosis and Hemostasis (ICTH). Assignment of numerals ceased in 1963 after the naming of Factor XIII. The names Fletcher Factor and

Fitzgerald Factor were given to further coagulation-related proteins, namely prekallikrein and high-molecular-weight kininogen, respectively.

Factors III and VI are unassigned, as thromboplastin was never identified, and actually turned out to consist of ten further factors, and accelerin was found to be activated Factor V.

Other species

All mammals have an extremely closely related blood coagulation process, using a combined cellular and serine protease process. In fact, it is possible for any mammalian coagulation factor to "cleave" its equivalent target in any other mammal. The only nonmammalian animal known to use serine proteases for blood coagulation is the horseshoe crab.

Chapter 3

Penetrating Trauma

Penetrating trauma

Acute penetrating trauma from a close-range shotgun blast injury to knee. Birdshot pellets are visible in the wound, within the shattered patella. The powder wad from the shotgun shell has been extracted from the wound, and is visible at the upper right of the image.

ICD-10 T14.1

Penetrating trauma is an injury that occurs when an object pierces the skin and enters a tissue of the body, creating an open wound. In blunt, or non-penetrating trauma, there may be an impact, but the skin is not necessarily broken. The penetrating object may remain in the tissues, come back out the way it entered, or pass through the tissues and exit from another area. An injury in which an object enters the body or a structure and passes all the way through is called a **perforating injury**, while *penetrating trauma* implies that the object does not pass through. Perforating trauma is associated with an **entrance wound** and an often larger **exit wound**.

Penetrating trauma can be caused by a foreign object or by fragments of a broken bone. Usually occurring in violent crime or armed combat, penetrating injuries are commonly caused by gunshots and stabbings.

Penetrating trauma can be serious because it can damage internal organs and presents a risk of shock and infection. The severity of the injury varies widely depending on the

body parts involved, the characteristics of the penetrating object, and the amount of energy transmitted to the tissues. Assessment may involve X-rays or CT scans, and treatment may involve surgery, for example to repair damaged structures or to remove foreign objects.

Mechanism

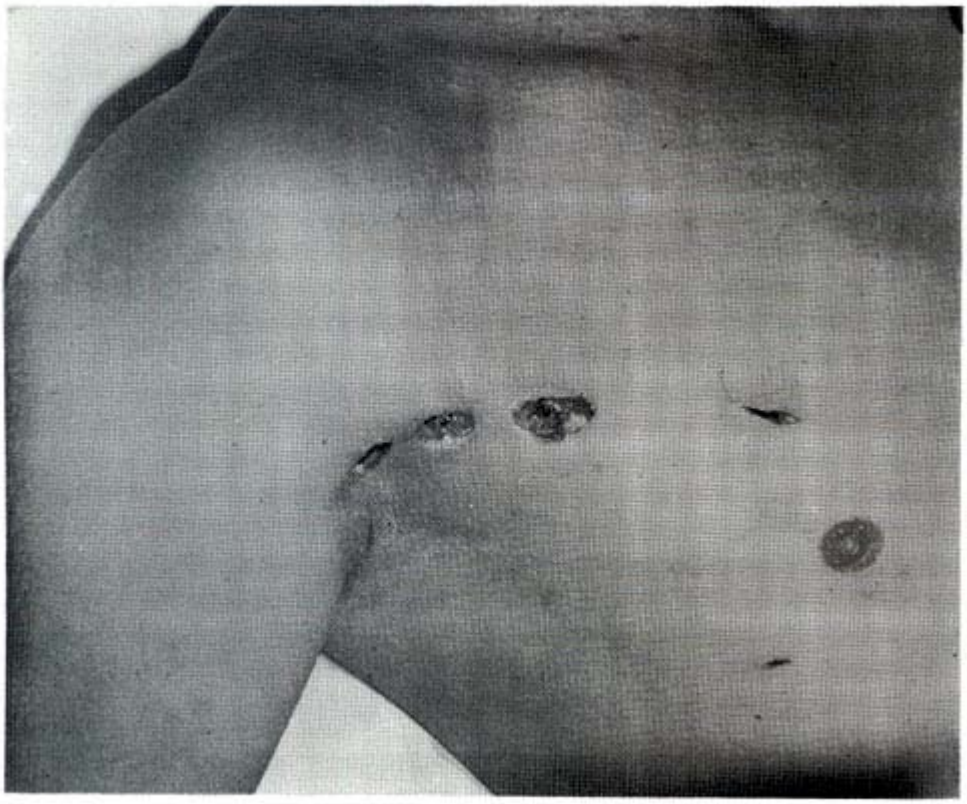


FIGURE 1.—Superficial bullet wounds of chest wall not involving bony structures.

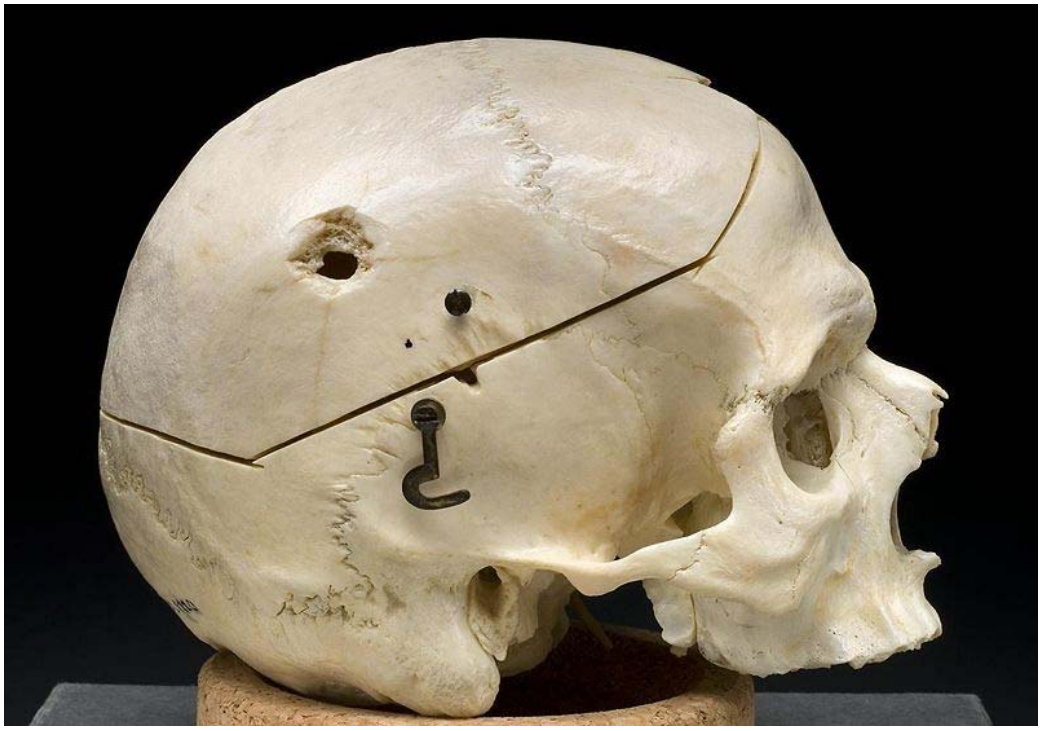
A gunshot wound

As a missile passes through tissue, it decelerates, dissipating and transferring kinetic energy to the tissues; this is what causes the injury. The velocity of the projectile is a more important factor than its mass in determining how much damage is done; kinetic energy increases with the square of the velocity. In addition to injury caused directly by the object that enters the body, penetrating injuries may be associated with secondary injuries, due for example to a blast injury. High-velocity objects are usually projectiles such as bullets from high-powered rifles, such as assault rifles or sniper rifles. Bullets classed as medium-velocity projectiles include those from handguns, shotguns, and submachine guns. Low-velocity items, such as knives, are usually propelled by a person's hand, and usually do damage only to the area that is directly contacted by the object. The space left by tissue that is destroyed by the penetrating object as it passes through forms a cavity; this is called permanent cavitation. In addition to causing damage to the tissues

they contact, medium- and high-velocity projectiles cause a secondary cavitation injury: as the object enters the body, it creates a pressure wave which forces tissue out of the way, creating a "temporary cavity" that can be much larger than the object itself. The tissues soon move back into place, eliminating the cavity, but the cavitation frequently does considerable damage first. Temporary cavitation can be especially damaging when it affects delicate tissues such as the brain, as occurs in penetrating head trauma.

The characteristics of the tissue injured also help determine the severity of the injury; for example, the denser the tissue, the greater the amount of energy transmitted to it. The path of a projectile can be estimated by imagining a line from the entrance wound to the exit wound, but the actual trajectory may vary due to ricochet or differences in tissue density.

Head



A skull with a gunshot wound

While penetrating head trauma accounts for only a small percentage of all traumatic brain injuries, it is associated with a high mortality rate, and only a third of people with penetrating head trauma survive long enough to arrive at a hospital. Injuries from firearms are the leading cause of TBI-related deaths. Penetrating head trauma can cause cerebral contusions and lacerations, intracranial hematomas, pseudoaneurysms, and arteriovenous fistulas. The prognosis for penetrating head injuries varies widely.

Penetrating facial trauma can pose a risk to the airway and breathing; airway obstruction can occur later due to swelling or bleeding. Penetrating eye trauma can cause the globe of

the eye to rupture or vitreous humor to leak from it, and presents a serious threat to eyesight.

Chest

Most penetrating injuries are chest wounds and have a mortality rate (death rate) of under 10%. Penetrating chest trauma can injure vital organs such as the heart and lungs and can interfere with breathing and circulation. Lung injuries that can be caused by penetrating trauma include pulmonary laceration (a cut or tear) pulmonary contusion (a bruise), hemothorax (an accumulation of blood in the chest cavity outside of the lung), pneumothorax (an accumulation of air in the chest cavity) and hemopneumothorax (accumulation of both blood and air). Sucking chest wounds and tension pneumothorax may result.

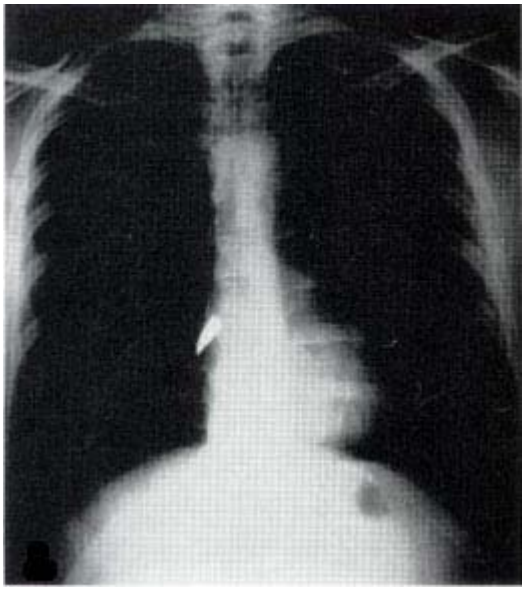
Penetrating trauma can also cause injuries to the heart and circulatory system. When the heart is punctured, it may bleed profusely into the chest cavity if the membrane around it {the pericardium) is significantly torn, or it may cause pericardial tamponade if the pericardium is not disrupted. In pericardial tamponade, blood escapes from the heart but is trapped within the pericardium, so pressure builds up between the pericardium and the heart, compressing the latter and interfering with its pumping. Fractures of the ribs commonly produce penetrating chest trauma when sharp bone ends pierce tissues.

Abdomen

Penetrating abdominal trauma (PAT) can be life threatening because abdominal organs, especially those in the retroperitoneal space, can bleed profusely, and the space can hold a great deal of blood. If the pancreas is injured, it may be further injured by its own secretions, in a process called *autodigestion*. Injuries of the liver, common because of the size and location of the organ, present a serious risk for shock because the liver tissue is delicate and has a large blood supply and capacity. The intestines, taking a large part of the lower abdomen, are also at risk of perforation.

People with penetrating abdominal trauma may have signs of hypovolemic shock (insufficient blood in the circulatory system) and peritonitis (an inflammation of the peritoneum, the membrane that lines the abdominal cavity). Penetration may abolish or diminish bowel sounds due to bleeding, infection, and irritation, and injuries to arteries may cause bruits (a distinctive sound similar to heart murmurs) to be audible. Percussion of the abdomen may reveal hyperresonance (indicating air in the abdominal cavity) or dullness (indicating a buildup of blood). The abdomen may be distended or tender, signs which indicate an urgent need for surgery.

Assessment and treatment



X-ray showing a bullet (white spot) in the heart

Assessment can be difficult because much of the damage is often internal and not visible. The patient is thoroughly examined. X-ray and CT scanning may be used to identify the type and location of potentially lethal injuries. Sometimes before an X-ray is performed on a person with penetrating trauma from a projectile, a paper clip is taped over entry and exit wounds to show their location on the film. The patient is given intravenous fluids to replace lost blood. Surgery may be required; impaled objects are secured into place so that they do not move and cause further injury, and they are removed in an operating room. Foreign bodies such as bullets may be removed, but they may also be left in place if the surgery necessary to get them out would cause more damage than would leaving them. Wounds are debrided to remove tissue that cannot survive and other material that presents risk for infection.

History

Ambroise Paré

Before the 17th century, medical practitioners poured hot oil into wounds in order to cauterize damaged blood vessels, but the French surgeon Ambroise Paré challenged the use of this method in 1545. Paré was the first to propose controlling bleeding using ligature.

During the American Civil War, chloroform was used during surgery to reduce pain and allow more time for operations. Due in part to the fact that sterile technique was not used in hospitals, infection was the leading cause of death for wounded soldiers.

In World War I, doctors began replacing patients' lost fluid with salt solutions. With World War II came the idea of blood banking, having quantities of donated blood available to replace lost fluids. The use of antibiotics also came into practice in World War II.

Chapter 4

Angiogenesis

Angiogenesis is the physiological process involving the growth of new blood vessels from pre-existing vessels. Though there has been some debate over terminology, vasculogenesis is the term used for spontaneous blood-vessel formation, and intussusception is the term for new blood vessel formation by splitting off existing ones.

Angiogenesis is a normal and vital process in growth and development, as well as in wound healing and in granulation tissue. However, it is also a fundamental step in the transition of tumors from a dormant state to a malignant one. The identification of an angiogenic diffusible factor derived from tumors was made initially by Greenblatt and Shubik in 1968.

Types

Sprouting angiogenesis

Sprouting angiogenesis was the first identified form of angiogenesis. It occurs in several well-characterized stages. First, biological signals known as angiogenic growth factors activate receptors present on endothelial cells present in pre-existing blood vessels. Second, the activated endothelial cells begin to release enzymes called proteases that degrade the basement membrane to allow endothelial cells to escape from the original (parent) vessel walls. The endothelial cells then proliferate into the surrounding matrix and form solid sprouts connecting neighboring vessels. As sprouts extend toward the source of the angiogenic stimulus, endothelial cells migrate in tandem, using adhesion molecules, the equivalent of cellular grappling hooks, called integrins. These sprouts then form loops to become a full-fledged vessel lumen as cells migrate to the site of angiogenesis. Sprouting occurs at a rate of several millimeters per day, and enables new vessels to grow across gaps in the vasculature. It is markedly different from splitting angiogenesis, however, because it forms entirely new vessels as opposed to splitting existing vessels.

Intussusceptive angiogenesis

Intussusception, also known as splitting angiogenesis, was first observed in neonatal rats. In this type of vessel formation, the capillary wall extends into the lumen to split a single vessel in two. There are four phases of intussusceptive angiogenesis. First, the two opposing capillary walls establish a zone of contact. Second, the endothelial cell junctions are reorganized and the vessel bilayer is perforated to allow growth factors and cells to penetrate into the lumen. Third, a core is formed between the two new vessels at the zone of contact that is filled with pericytes and myofibroblasts. These cells begin laying collagen fibers into the core to provide an extracellular matrix for growth of the vessel lumen. Finally, the core is fleshed out with no alterations to the basic structure. Intussusception is important because it is a reorganization of existing cells. It allows a vast increase in the number of capillaries without a corresponding increase in the number of endothelial cells. This is especially important in embryonic development as there are not enough resources to create a rich microvasculature with new cells every time a new vessel develops.

Modern terminology of angiogenesis

Besides the differentiation between “Sprouting angiogenesis” and “Intussusceptive angiogenesis” there exists the today more common differentiation between the following types of angiogenesis:

Vasculogenesis – Formation of vascular structures from circulating or tissue-resident endothelial stem cells (angioblasts), which proliferate into *de novo* endothelial cells. This form particularly relates to the embryonal development of the vascular system.

Angiogenesis – Formation of thin-walled endothelium-lined structures with muscular smooth muscle wall and pericytes (fibrocytes). This form plays an important role during the adult life span, also as "repair mechanism" of damaged tissues.

Arteriogenesis – Formation of medium-sized blood vessels possessing tunica media plus adventitia.

Because it turned out that even this differentiation is not a sharp one, today quite often the term “angiogenesis” is used summarizing all different types and modifications of arterial vessel growth.

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Angiogenesis as a therapeutic target

Angiogenesis may be a target for combating diseases characterized by either poor vascularisation or abnormal vasculature. Application of specific compounds that may inhibit or induce the creation of new blood vessels in the body may help combat such diseases. The presence of blood vessels where there should be none may affect the mechanical properties of a tissue, increasing the likelihood of failure. The absence of blood vessels in a repairing or otherwise metabolically active tissue may inhibit repair or other essential functions. Several diseases, such as ischemic chronic wounds, are the result of failure or insufficient blood vessel formation and may be treated by a local expansion of blood vessels, thus bringing new nutrients to the site, facilitating repair. Other diseases, such as age-related macular degeneration, may be created by a local expansion of blood vessels, interfering with normal physiological processes.

The modern clinical application of the principle of angiogenesis can be divided into two main areas: anti-angiogenic therapies, which angiogenic research began with, and pro-angiogenic therapies. Whereas anti-angiogenic therapies are being employed to fight cancer and malignancies, which require an abundance of oxygen and nutrients to proliferate, pro-angiogenic therapies are being explored as options to treat cardiovascular diseases, the number one cause of death in the Western world. One of the first applications of pro-angiogenic methods in humans was a German trial using fibroblast growth factor 1 (FGF-1) for the treatment of coronary artery disease. Clinical research in therapeutic angiogenesis is ongoing for a variety of atherosclerotic diseases, like coronary heart disease, peripheral arterial disease, wound healing disorders, etc..

Also, regarding the mechanism of action, pro-angiogenic methods can be differentiated into three main categories: gene-therapy, targeting genes of interest for amplification or inhibition; protein-therapy, which primarily manipulates angiogenic growth factors like FGF-1 or vascular endothelial growth factor, VEGF; and cell-based therapies, which involve the implantation of specific cell types.

There are still serious, unsolved problems related to gene therapy. Difficulties include effective integration of the therapeutic genes into the genome of target cells, reducing the risk of an undesired immune response, potential toxicity, immunogenicity, inflammatory responses, and oncogenesis related to the viral vectors used in implanting genes and the sheer complexity of the genetic basis of angiogenesis. The most commonly-occurring disorders in humans, such as heart disease, high blood pressure, diabetes and Alzheimer's disease, are most likely caused by the combined effects of variations in many genes, and, thus, injecting a single gene may not be significantly beneficial in such diseases.

In contrast, pro-angiogenic protein therapy uses well-defined, precisely-structured proteins, with previously-defined optimal doses of the individual protein for disease states, and with well-known biological effects. On the other hand, an obstacle of protein therapy is the mode of delivery. Oral, intravenous, intra-arterial, or intramuscular routes of protein administration are not always as effective, as the therapeutic protein may be metabolized or cleared before it can enter the target tissue. Cell-based pro-angiogenic therapies are still early stages of research, with many open questions regarding best cell types and dosages to use.

Mechanical stimulation

Mechanical stimulation of angiogenesis is not well characterized. There is a significant amount of controversy with regard to shear stress acting on capillaries to cause angiogenesis, although current knowledge suggests that increased muscle contractions may increase angiogenesis. This may be due to an increase in the production of nitric oxide during exercise. Nitric oxide results in vasodilation of blood vessels.

Chemical stimulation

Chemical stimulation of angiogenesis is performed by various angiogenic proteins, including several growth factors.

Overview

Stimulator	Mechanism
FGF	Promotes proliferation & differentiation of endothelial cells, smooth muscle cells, and fibroblasts
VEGF	Affects permeability
VEGFR and NRP-1	Integrate survival signals
Ang1 and Ang2	Stabilize vessels
PDGF (BB-homodimer) and PDGFR	recruit smooth muscle cells
TGF-β, endoglin and TGF-β receptors	↑extracellular matrix production
MCP-1	
Integrins $\alpha_V\beta_3$, $\alpha_V\beta_5$ (?) and $\alpha_5\beta_1$	Bind matrix macromolecules and proteinases
VE-cadherin and CD31	endothelial junctional molecules
ephrin	Determine formation of arteries or veins
plasminogen activators	remodels extracellular matrix, releases and activates growth factors
plasminogen activator inhibitor-1	stabilizes nearby vessels
eNOS and COX-2	

AC133	regulates angioblast differentiation
Id1/Id3	Regulates endothelial transdifferentiation

FGF

The fibroblast growth factor (FGF) family with its prototype members FGF-1 (acidic FGF) and FGF-2 (basic FGF) consists to date of at least 22 known members. Most are single-chain peptides of 16-18 kDa and display high affinity to heparin and heparan sulfate. In general, FGFs stimulate a variety of cellular functions by binding to cell surface FGF-receptors in the presence of heparin proteoglycans. The FGF-receptor family is composed of seven members, and all the receptor proteins are single-chain receptor tyrosine kinases that become activated through autophosphorylation induced by a mechanism of FGF-mediated receptor dimerization. Receptor activation gives rise to a signal transduction cascade that leads to gene activation and diverse biological responses, including cell differentiation, proliferation, and matrix dissolution, thus initiating a process of mitogenic activity critical for the growth of endothelial cells, fibroblasts, and smooth muscle cells. **FGF-1**, unique among all 22 members of the FGF family, can bind to all seven FGF-receptor subtypes, making it the broadest-acting member of the FGF family, and a potent mitogen for the diverse cell types needed to mount an angiogenic response in damaged (hypoxic) tissues, where upregulation of FGF-receptors occurs. FGF-1 stimulates the proliferation and differentiation of all cell types necessary for building an arterial vessel, including endothelial cells and smooth muscle cells; this fact *distinguishes FGF-1 from other pro-angiogenic growth factors*, such as vascular endothelial growth factor (VEGF), which primarily drives the formation of new capillaries.

Until now (2007), three human clinical trials have been successfully completed with FGF-1, in which the angiogenic protein was injected directly into the damaged heart muscle. Also, one additional human FGF-1 trial has been completed to promote wound healing in diabetics with chronic wounds.

Besides FGF-1, one of the most important functions of fibroblast growth factor-2 (FGF-2 or bFGF) is the promotion of endothelial cell proliferation and the physical organization of endothelial cells into tube-like structures, thus promoting angiogenesis. FGF-2 is a more potent angiogenic factor than VEGF or PDGF (platelet-derived growth factor); however, it is less potent than FGF-1. As well as stimulating blood vessel growth, aFGF (FGF-1) and bFGF (FGF-2) are important players in wound healing. They stimulate the proliferation of fibroblasts and endothelial cells that give rise to angiogenesis and developing granulation tissue; both increase blood supply and fill up a wound space/cavity early in the wound-healing process.

VEGF

Vascular endothelial growth factor (VEGF) has been demonstrated to be a major contributor to angiogenesis, increasing the number of capillaries in a given network. Initial *in vitro* studies demonstrated bovine capillary endothelial cells will proliferate and

show signs of tube structures upon stimulation by VEGF and bFGF, although the results were more pronounced with VEGF. Upregulation of VEGF is a major component of the physiological response to exercise and its role in angiogenesis is suspected to be a possible treatment in vascular injuries. *In vitro* studies clearly demonstrate that VEGF is a potent stimulator of angiogenesis because, in the presence of this growth factor, plated endothelial cells will proliferate and migrate, eventually forming tube structures resembling capillaries. VEGF causes a massive signaling cascade in endothelial cells. Binding to VEGF receptor-2 (VEGFR-2) starts a tyrosine kinase signaling cascade that stimulates the production of factors that variously stimulate vessel permeability (eNOS, producing NO), proliferation/survival (bFGF), migration (ICAMs/VCAMs/MMPs) and finally differentiation into mature blood vessels. Mechanically, VEGF is upregulated with muscle contractions as a result of increased blood flow to affected areas. The increased flow also causes a large increase in the mRNA production of VEGF receptors 1 and 2. The increase in receptor production means muscle contractions could cause upregulation of the signaling cascade relating to angiogenesis. As part of the angiogenic signaling cascade, NO is widely considered to be a major contributor to the angiogenic response because inhibition of NO significantly reduces the effects of angiogenic growth factors. However, inhibition of NO during exercise does not inhibit angiogenesis, indicating there are other factors involved in the angiogenic response.

Angiopoietins

The angiopoietins, Ang1 and Ang2, are required for the formation of mature blood vessels, as demonstrated by mouse knock out studies. Ang1 and Ang2 are protein growth factors which act by binding their receptors, Tie-1 and Tie-2; while this is somewhat controversial, it seems that cell signals are transmitted mostly by Tie-2; though some papers show physiologic signaling via Tie-1 as well. These receptors are tyrosine kinases. Thus, they can initiate cell signaling when ligand binding causes a dimerization that initiates phosphorylation on key tyrosines.

MMP

Another major contributor to angiogenesis is matrix metalloproteinase (MMP). MMPs help degrade the proteins that keep the vessel walls solid. This proteolysis allows the endothelial cells to escape into the interstitial matrix as seen in sprouting angiogenesis. Inhibition of MMPs prevents the formation of new capillaries. These enzymes are highly regulated during the vessel formation process because destruction of the extracellular matrix would decrease the integrity of the microvasculature.

Dll4

Delta-like ligand 4 (DII4) is a recently discovered protein with an important negative regulatory effect on angiogenesis. Dll4 is a transmembrane ligand, for the notch family of receptors.

Chemical inhibition

Angiogenesis inhibitor can be endogenous or come from outside as drug or a dietary component.

Applications

Tumor angiogenesis

Cancer cells are cells that have lost their ability to divide in a controlled fashion. A tumor consists of a population of rapidly dividing and growing cancer cells. Mutations rapidly accrue within the population. These mutations (variation) allow the cancer cells (or sub-populations of cancer cells within a tumor) to develop drug resistance and escape therapy. Tumors cannot grow beyond a certain size, generally 1–2 mm^3 , due to a lack of oxygen and other essential nutrients.

Tumors induce blood vessel growth (angiogenesis) by secreting various growth factors (e.g. VEGF). Growth factors such as bFGF and VEGF can induce capillary growth into the tumor, which some researchers suspect supply required nutrients, allowing for tumor expansion. In 2007, it was discovered that cancerous cells stop producing the anti-VEGF enzyme PKG. In normal cells (but not in cancerous ones), PKG apparently limits beta-catenin, which solicits angiogenesis. Other clinicians believe angiogenesis really serves as a waste pathway, taking away the biological end products secreted by rapidly dividing cancer cells. In either case, angiogenesis is a necessary and required step for transition from a small harmless cluster of cells, often said to be about the size of the metal ball at the end of a ball-point pen, to a large tumor. Angiogenesis is also required for the spread of a tumor, or metastasis. Single cancer cells can break away from an established solid tumor, enter the blood vessel, and be carried to a distant site, where they can implant and begin the growth of a secondary tumor. Evidence now suggests the blood vessel in a given solid tumor may, in fact, be mosaic vessels, composed of endothelial cells and tumor cells. This mosaicity allows for substantial shedding of tumor cells into the vasculature, possibly contributing to the appearance of circulating tumor cells in the peripheral blood of patients with malignancies. The subsequent growth of such metastases will also require a supply of nutrients and oxygen and a waste disposal pathway.

Endothelial cells have long been considered genetically more stable than cancer cells. This genomic stability confers an advantage to targeting endothelial cells using antiangiogenic therapy, compared to chemotherapy directed at cancer cells, which rapidly mutate and acquire 'drug resistance' to treatment. For this reason, endothelial cells are thought to be an ideal target for therapies directed against them. Recent studies by Klagsbrun, et al. have shown, however, that endothelial cells growing within tumors do carry genetic abnormalities. Thus, tumor vessels have the theoretical potential for developing acquired resistance to drugs. This is a new area of angiogenesis research being actively pursued.

Two independent studies published in the journal Nature in 2010 November confirmed the ability of tumors to make their own blood vessels. When one group found that tumor stem cells could make their own blood vessels and avastin could not inhibit their early differentiation, the other group showed that selective targeting of endothelial cells generated by tumor-derived stem cells in mouse xenografts resulted in tumour reduction. These studies done in glioblastoma model may have implications in other tumors.

Formation of tumor blood vessels

Tumor blood vessels have perivascular detachment, vessel dilation, and irregular shape. It is believed tumor blood vessels are not smooth like normal tissues, and are not ordered sufficiently to give oxygen to all of the tissues. Endothelial precursor cells are organized from bone marrow, which are then integrated into the growing blood vessels. Then the endothelial cells differentiate and migrate into perivascular space, to form tumour cells. VEGF plays a crucial role in the formation of blood vessels that lead to tumor growth, which allows the vessel to expand. It is called sprouting angiogenesis.

Angiogenesis research is a cutting-edge field in cancer research, and recent evidence also suggests traditional therapies, such as radiation therapy, may actually work in part by targeting the genomically stable endothelial cell compartment, rather than the genomically unstable tumor cell compartment. New blood vessel formation is a relatively fragile process, subject to disruptive interference at several levels. In short, the therapy is the selection agent which is being used to kill a cell compartment. Tumor cells evolve resistance rapidly due to rapid generation time (days) and genomic instability (variation), whereas endothelial cells are a good target because of a long generation time (months) and genomic stability (low variation).

This is an example of selection in action at the cellular level, using a selection pressure to target and differentiate between varying populations of cells. The end result is the extinction of one species or population of cells (endothelial cells), followed by the collapse of the ecosystem (the tumor) due either to nutrient deprivation or self-pollution from the destruction of necessary waste pathways.

Angiogenesis-based tumor therapy relies on natural and synthetic angiogenesis inhibitors like angiostatin, endostatin and tumstatin. These are proteins that mainly originate as specific fragments of pre-existing structural proteins like collagen or plasminogen.

Recently, the first FDA-approved therapy targeted at angiogenesis in cancer came on the market in the US. This is a monoclonal antibody directed against an isoform of VEGF. The commercial name of this antibody is Avastin, and the therapy has been approved for use in colorectal cancer in combination with established chemotherapy.

Angiogenesis for cardiovascular disease

Angiogenesis represents an excellent therapeutic target for the treatment of cardiovascular disease. It is a potent, physiological process that underlies the natural

manner in which our bodies respond to a diminution of blood supply to vital organs, namely the production of new collateral vessels to overcome the ischemic insult. A large number of preclinical studies have been performed with protein-, gene- and cell-based therapies in animal models of cardiac ischemia, as well as models of peripheral artery disease. Reproducible and credible successes in these early animal studies led to high enthusiasm that this new therapeutic approach could be rapidly translated to a clinical benefit for millions of patients in the Western world suffering from these disorders. A decade of clinical testing both gene- and protein-based therapies designed to stimulate angiogenesis in underperfused tissues and organs, however, has led from one disappointment to another. Although all of these preclinical readouts, which offered great promise for the transition of angiogenesis therapy from animals to humans, were in one fashion or another, incorporated into early stage clinical trials, the FDA has, to date (2007), insisted that the primary endpoint for approval of an angiogenic agent must be an improvement in exercise performance of treated patients.

If one reviews in detail the various published angiogenesis clinical trials, it can be realized that most of these trials had success in achieving various secondary or supportive endpoints, but failed when attempting to demonstrate a statistically significant improvement in exercise performance, typically done by a treadmill exercise test. Perhaps the greatest reason for these trials' failure to achieve success is the high occurrence of the "placebo effect" in studies employing treadmill exercise test readout. Thus, even though a majority of the treated patients in these trials experience relief of such clinical symptoms as chest pain (angina), and generally performed better on most efficacy readouts, there were enough "responders" in the blinded placebo groups to render the trial inconclusive. In addition to the placebo effect, more recent animal studies have also highlighted various factors that may inhibit an angiogenesis response, including certain drugs, smoking, and hypercholesterolemia.

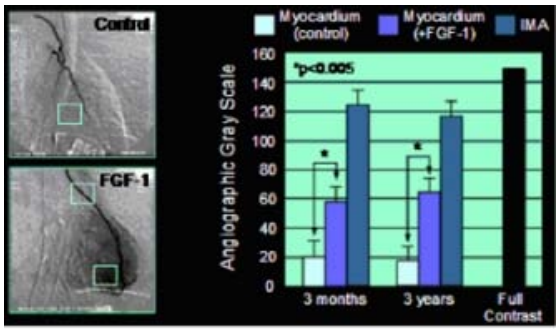
Although shown to be relatively safe therapies, not one angiogenic therapeutic has yet made it through the gauntlet of clinical testing required for drug approval. By capitalizing on the large database of what did and did not work in previous clinical trials, results from more recent studies with redesigned clinical protocols give renewed hope that angiogenesis therapy will be a treatment choice for sufferers of cardiovascular disease resulting from occluded and/or stenotic vessels.

Early clinical studies with protein-based therapeutics largely focused on the intravenous or intracoronary administration of a particular growth factor to stimulate angiogenesis in the affected tissue or organ. Most of these trials did not achieve statistically significant improvements in their clinical endpoints. This ultimately led to an abandonment of this approach and a widespread belief in the field that protein therapy, especially with a single agent, was not a viable option to treat ischemic cardiovascular disease. However, the failure of gene- or cell-based therapy to deliver, as of yet, a suitable treatment choice for diseases resulting from poor blood flow, has led to a resurgence of interest in returning to protein-based therapy to stimulate angiogenesis.

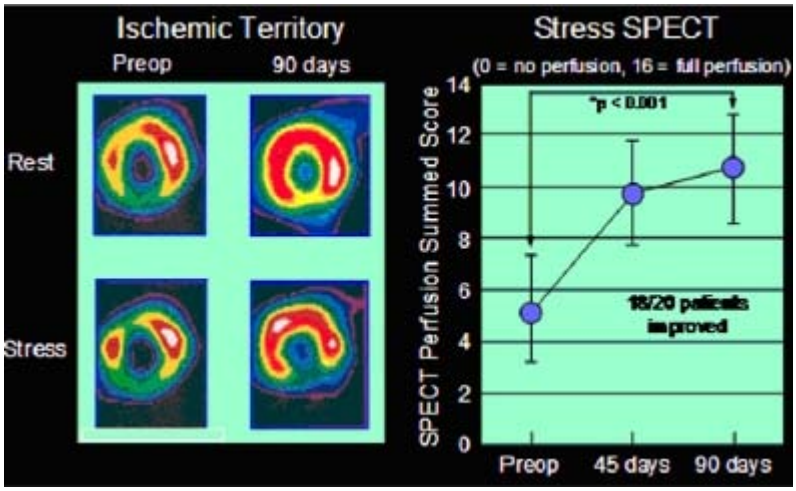
These failures suggested that either these are the wrong molecular targets to induce neovascularization, that they can only be effectively used if formulated and administered correctly, or that their presentation in the context of the overall cellular microenvironment may play a vital role in their utility. It may be necessary to present these proteins in a way that mimics natural signaling events, including the concentration, spatial and temporal profiles, and their simultaneous or sequential presentation with other appropriate factors.

Lessons learned from earlier protein-based studies, which indicated that intravenous or intracoronary delivery of the protein was not efficacious, have led to completed and ongoing clinical trials in which the angiogenic protein is injected directly into the beating ischemic heart.

Such localized administration of the potent angiogenic growth factor, human FGF-1, has recently given promising results in clinical trials in no-option heart patients. Angiogenesis was documented by angiographically visible “blushing”, and functional exercise tests were also performed on a subset of patients. The attractiveness of protein therapy is that large amounts of the therapeutic agent can be injected into the ischemic area of interest, to pharmacologically start the process of blood vessel growth and collateral arteries formation. In addition, from pharmacokinetic data collected from the recent FGF-1 studies in the human heart, it appears that FGF-1, once it exits the heart, is cleared in less than three hours from the circulation. This would presumably prevent FGF-1 from stimulating unwanted angiogenesis in other tissues of the bodies where it could potentially cause harm, such as the retina and in the kidneys. No serious adverse events have yet to be noted in any of the completed or ongoing clinical trials in which the FGF-1 protein is used as the therapeutic agent to stimulate angiogenesis.



Left: Angiographic "blushing" following FGF-1 injection into the human heart. Right, measurements of pixel density in angiograms ("gray-value-analysis") indicating a threefold increase in vessel density in the treated human myocardium (3 months and 3 years).



Improvement in myocardial perfusion (blood supply) after FGF-1 treatment as demonstrated by single photon emission computed tomography (SPECT) imaging.

Exercise

Angiogenesis is generally associated with aerobic exercise and endurance exercise. While arteriogenesis produces network changes that allow for a large increase in the amount of total flow in a network, angiogenesis causes changes that allow for greater nutrient delivery over a long period of time. Capillaries are designed to provide maximum nutrient delivery efficiency, so an increase in the number of capillaries allows the network to deliver more nutrients in the same amount of time. A greater number of capillaries also allows for greater oxygen exchange in the network. This is vitally important to endurance training, because it allows a person to continue training for an extended period of time. No experimental evidence exists, though, to suggest increased capillarity is required in endurance exercise to increase the maximum oxygen delivery.

Macular degeneration

Overexpression of VEGF causes increased permeability in blood vessels in addition to stimulating angiogenesis. In wet macular degeneration, VEGF causes proliferation of capillaries into the retina. Since the increase in angiogenesis also causes edema, blood and other retinal fluids leak into the retina, causing loss of vision. A novel treatment of this disease is to use a VEGF inhibiting siRNA to stop the main signaling cascade for angiogenesis.

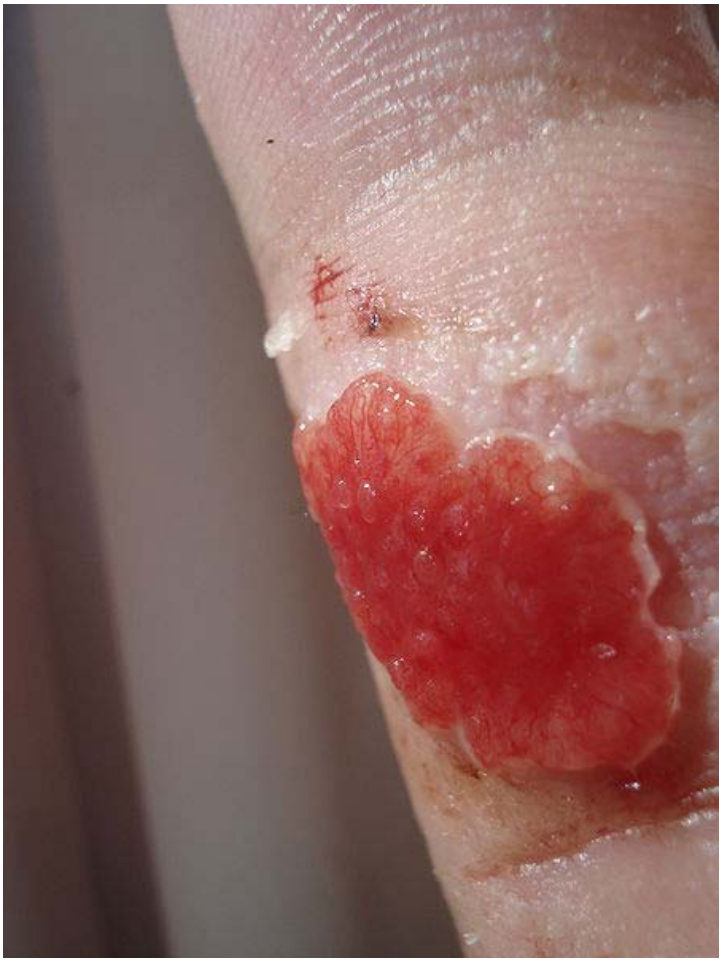
Chapter 5

Granulation Tissue and Epidermal Growth Factor

Granulation tissue

Granulation tissue is the perfused, fibrous connective tissue that replaces a fibrin clot in healing wounds. Granulation tissue typically grows from the base of a wound and is able to fill wounds of almost any size it heals. In addition, it is also found in ulcers like oesophageal ulcer.

Appearance



Example of granulation tissue from a cut on a finger with "proud flesh".

During the proliferative phase of wound healing, granulation tissue is:

- light red or dark pink in color, being perfused (permeated) with new capillary loops or "buds";
- soft to the touch;
- moist; and
- bumpy (granular) in appearance.

Structure

Granulation tissue is composed of tissue matrix supporting a variety of cell types, most of which can be associated with one of the following functions:

- extracellular matrix,
- immune system, or
- vascularisation.

An excess of granulation tissue (caro luxurians) is informally referred to as "proud flesh."

Extracellular matrix

The extracellular matrix of granulation tissue is created and modified by fibroblasts. Initially, it consists of a network of Type III collagen, a weaker form of the structural protein that can be produced rapidly. This is later replaced by the stronger, long-stranded Type I collagen, as evidenced in scar tissue.

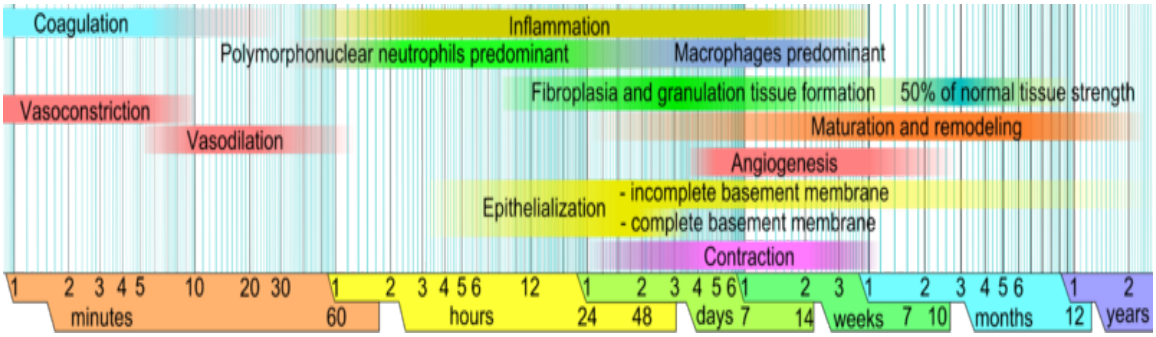
Immunity

The main immune cells active in the tissue are macrophages and neutrophils, although other leukocytes are also present. These work to phagocytize old or damaged tissue, and protect the healing tissue from pathogenic infection. This is necessary both to aid the healing process and to protect against invading pathogens, as the wound often does not have an effective skin barrier to act as a first line of defense.

Vascularization

It is necessary for a network of blood vessels to be established as soon as possible to provide the growing tissue with nutrients, to take away cellular wastes, and transport new leukocytes to the area. Fibroblasts, the main cells that deposit granulation tissue, depend on oxygen to proliferate and lay down the new extracellular matrix.

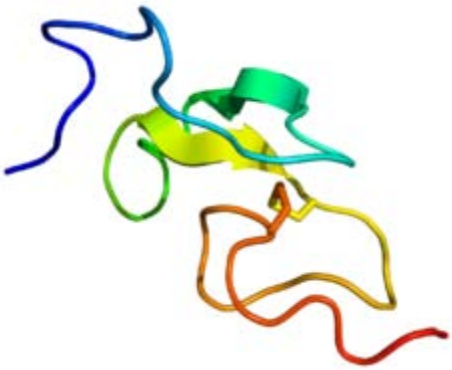
In vascularisation, also called angiogenesis, endothelial cells quickly grow into the tissue from older, intact blood vessels. These branch out in a systematic way, forming anastomoses with other vessels.



Approximate times of the different phases of wound healing, with substantial variation depending on wound size and healing conditions. *Granulation tissue formation* is seen in green box at days to weeks.

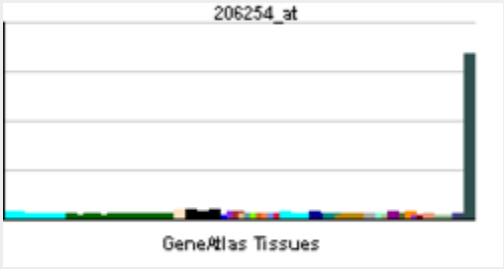
Epidermal growth factor

Epidermal growth factor (beta-urogastrone)



Rainbow colored NMR structure (N-terminus = blue, C-terminus = red) of the mouse epidermal growth factor.

Identifiers	
Symbols	EGF; URG
External IDs	OMIM: 131530 MGI: 95290 HomoloGene: 1483 GeneCards: EGF Gene
RNA expression pattern	



More reference expression data

Orthologs		
Species	**Human**	**Mouse**
Entrez	1950	13645
Ensembl	ENSG00000138798	ENSMUSG00000028017
UniProt	P01133	Q3UWD7
RefSeq (mRNA)	NM_001963	NM_010113
RefSeq (protein)	NP_001954	NP_034243
Location (UCSC)	Chr 4: 111.05 - 111.15 Mb	Chr 3: 129.67 - 129.75 Mb
PubMed search		

Epidermal growth factor or **EGF** is a growth factor that plays an important role in the regulation of cell growth, proliferation, and differentiation by binding to its receptor EGFR. Human EGF is a 6045-Da protein with 53 amino acid residues and three intramolecular disulfide bonds.

History

The discovery of EGF won Stanley Cohen of Vanderbilt University a Nobel Prize in Physiology and Medicine in 1986 and was patented for cosmetic use by Greg Brown in 1989.

Function

EGF results in cellular proliferation, differentiation, and survival. EGF is a low-molecular-weight polypeptide first purified from the mouse submandibular gland, but

since then found in many human tissues including submandibular gland, parotid gland. Salivary EGF, which seems also regulated by dietary inorganic iodine, also plays an important physiological role in the maintenance of oro-esophageal and gastric tissue integrity. The biological effects of salivary EGF include healing of oral and gastroesophageal ulcers, inhibition of gastric acid secretion, stimulation of DNA synthesis as well as mucosal protection from intraluminal injurious factors such as gastric acid, bile acids, pepsin, and trypsin and to physical, chemical and bacterial agents.

Biological Sources

Epidermal growth factor can be found in human platelets, macrophages, urine, saliva, milk, and plasma.

Mechanism

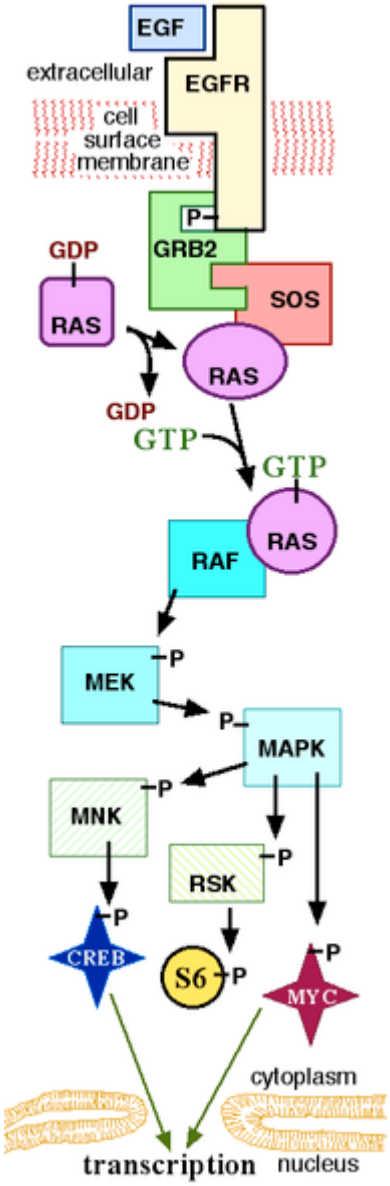


Diagram showing key components of the MAPK/ERK pathway. In the diagram, "P" represents phosphate. Note EGF at the very top.

EGF acts by binding with high affinity to epidermal growth factor receptor (EGFR) on the cell surface and stimulating the intrinsic protein-tyrosine kinase activity of the receptor. The tyrosine kinase activity, in turn, initiates a signal transduction cascade that results in a variety of biochemical changes within the cell - a rise in intracellular calcium levels, increased glycolysis and protein synthesis, and increases in the expression of certain genes including the gene for EGFR - that ultimately lead to DNA synthesis and cell proliferation.

EGF-family

EGF is the founding member of the EGF-family of proteins. Members of this protein family have highly similar structural and functional characteristics. Besides EGF itself other family members include:

- Heparin-binding EGF-like growth factor (HB-EGF)
- transforming growth factor-α (TGF-α)
- Amphiregulin (AR)
- Epiregulin (EPR)
- Epigen
- Betacellulin (BTC)
- neuregulin-1 (NRG1)
- neuregulin-2 (NRG2)
- neuregulin-3 (NRG3)
- neuregulin-4 (NRG4).

All family members contain one or more repeats of the conserved amino acid sequence:

$CX_7CX_{4-5}CX_{10-13}CXCX_8GXRC$

Where **X** represents any amino acid.

This sequence contains 6 cysteine residues that form three intramolecular disulfide bonds. Disulfide bond formation generates three structural loops that are essential for high-affinity binding between members of the EGF-family and their cell-surface receptors.

EGF therapy

Because of the increased risk of cancer by EGF, inhibiting it decreases cancer risk. Such medications are so far mainly based on inhibiting the EGF receptor. Monoclonal antibodies are potential substances for this purpose.

Interactions

Epidermal growth factor has been shown to interact with Epidermal growth factor receptor and PIK3R2.

Chapter 6

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (**VEGF**) is a signal protein produced by cells that stimulates the growth of new blood vessels. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate.

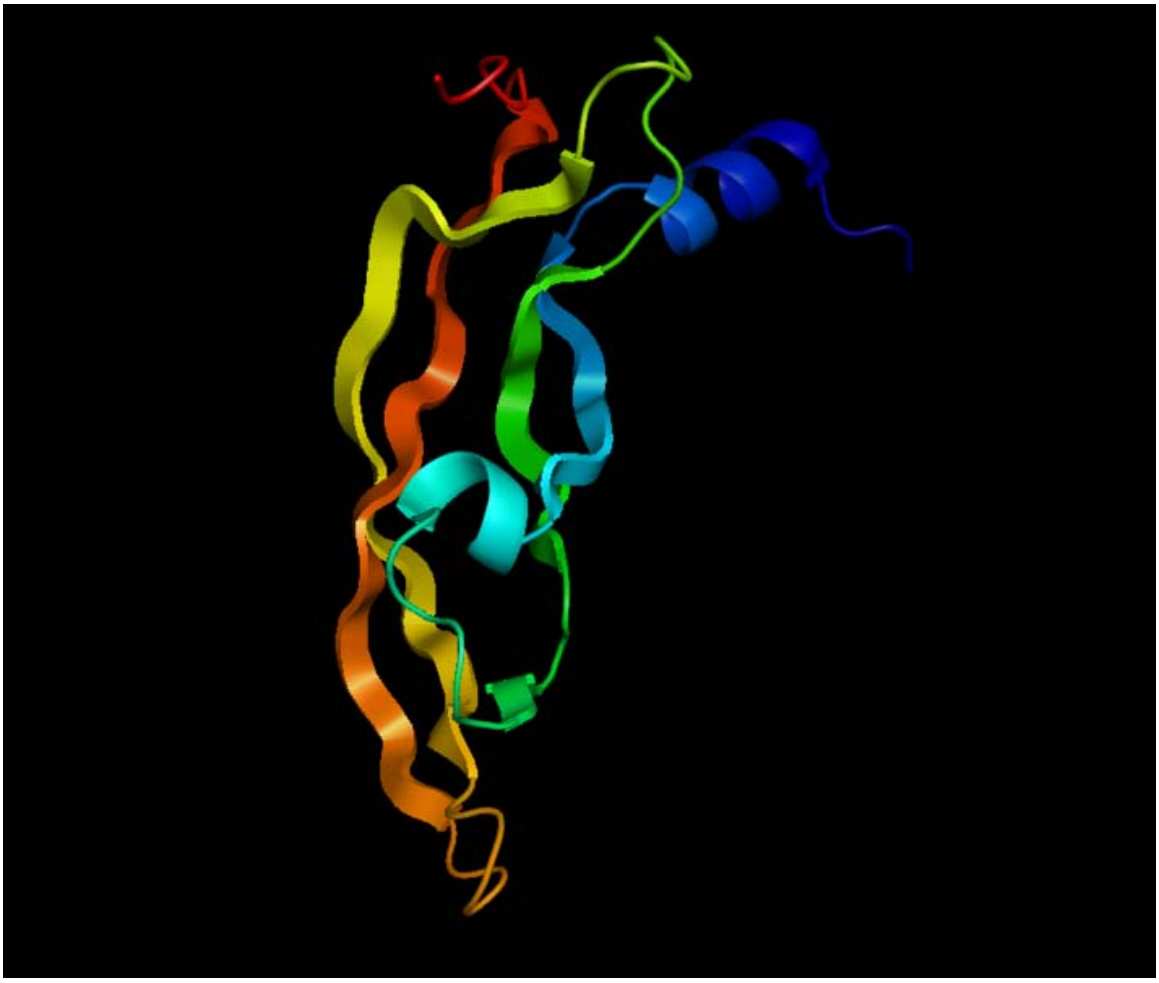
VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels.

When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and metastasize. Overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body. Drugs such as bevacizumab can inhibit VEGF and control or slow those diseases.

VEGF is a sub-family of growth factors, to be specific, the platelet-derived growth factor family of cystine-knot growth factors. They are important signaling proteins involved in both vasculogenesis (the *de novo* formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature).

Classification

The most important member is VEGF-A. Other members are Placenta growth factor (PlGF), VEGF-B, VEGF-C and VEGF-D. The latter ones were discovered later than VEGF-A, and, before their discovery, VEGF-A was called just VEGF.



Crystal structure of Vammin, a VEGF-F from a snake venom

A number of VEGF-related proteins have also been discovered encoded by viruses (VEGF-E) and in the venom of some snakes (VEGF-F).

Comparison

Type	Function

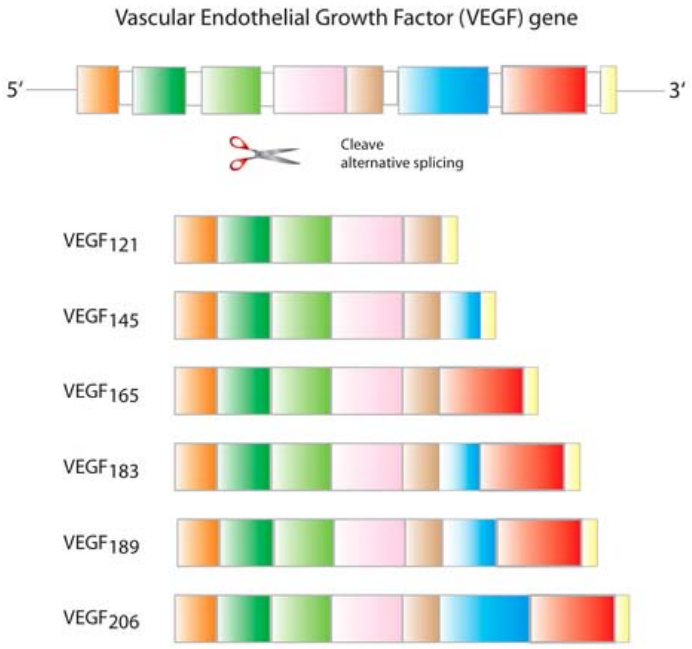
VEGF-A	• Angiogenesis
	  ○ ↑ Migration of endothelial cells
	  ○ ↑ mitosis of endothelial cells
	  ○ ↑ Methane monooxygenase activity
	  ○ ↑ αvβ3 activity
	  ○ creation of blood vessel lumen
	  ○ creates fenestrations
	• Chemotactic for macrophages and granulocytes
	• Vasodilation (indirectly by NO release)

VEGF-	Embryonic angiogenesis

B	
VEGF-C	Lymphangiogenesis
VEGF-D	Needed for the development of lymphatic vasculature surrounding lung bronchioles
PlGF	Important for Vasculogenesis, Also needed for angiogenesis during ischemia, inflammation, wound healing, and cancer.

Activity of **VEGF-A**, as its name implies, has been studied mostly on cells of the vascular endothelium, although it does have effects on a number of other cell types (e.g., stimulation monocyte/macrophage migration, neurons, cancer cells, kidney epithelial cells). *In vitro*, VEGF-A has been shown to stimulate endothelial cell mitogenesis and cell migration. VEGF-A is also a vasodilator and increases microvascular permeability and was originally referred to as vascular permeability factor.

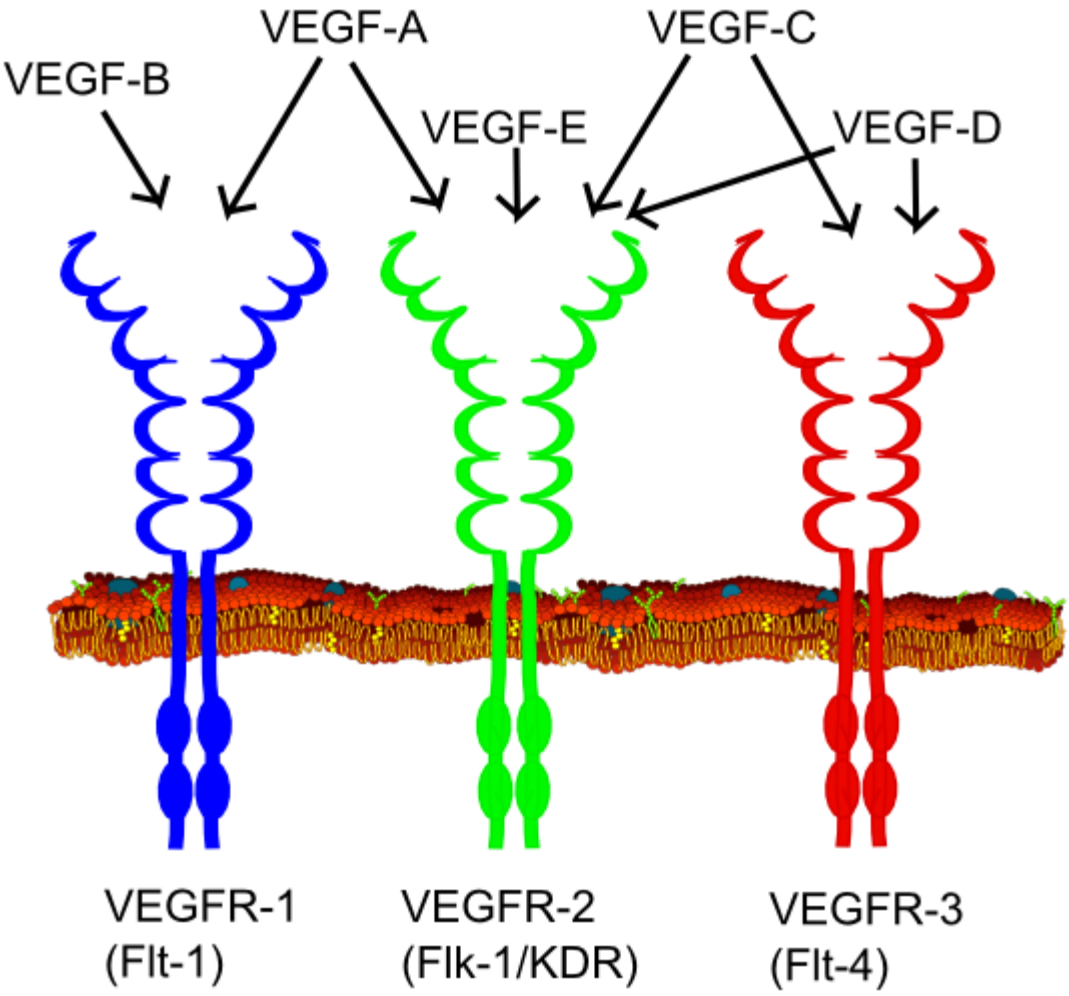
Alternative classification



Schematic representation of The different isoforms of human VEGF

The broad term 'VEGF' covers a number of proteins from two families, that result from alternate splicing of mRNA from a single, 8-exon, *VEGF* gene. The two different

families are referred to according to their terminal exon (exon 8) splice site - the proximal splice site (denoted $VEGF_{xxx}$) or distal splice site ($VEGF_{xxx}b$). In addition, alternate splicing of exon 6 and 7 alters their heparin-binding affinity, and amino acid number (in humans: $VEGF_{121}$, $VEGF_{121}b$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{165}b$, $VEGF_{189}$, $VEGF_{206}$; the rodent orthologs of these proteins contain one fewer amino acid). These domains have important functional consequences for the VEGF splice variants, as the terminal (exon 8) splice site determines whether the proteins are pro-angiogenic (proximal splice site, expressed during angiogenesis) or anti-angiogenic (distal splice site, expressed in normal tissues). In addition, inclusion or exclusion of exons 6 and 7 mediate interactions with heparan sulfate proteoglycans (HSPGs) and neuropilin co-receptors on the cell surface, enhancing their ability to bind and activate the VEGF receptors (VEGFRs).



Types of VEGF and their VEGF receptors.

Mechanism

All members of the VEGF family stimulate cellular responses by binding to tyrosine kinase receptors (the VEGFRs) on the cell surface, causing them to dimerize and become activated through transphosphorylation, although to different sites, times and extents. The VEGF receptors have an extracellular portion consisting of 7 immunoglobulin-like domains, a single transmembrane spanning region, and an intracellular portion containing a split tyrosine-kinase domain. VEGF-A binds to VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). VEGFR-2 appears to mediate almost all of the known cellular responses to VEGF. The function of VEGFR-1 is less well-defined, although it is thought to modulate VEGFR-2 signaling. Another function of VEGFR-1 may be to act as a dummy/decoy receptor, sequestering VEGF from VEGFR-2 binding (this appears to be particularly important during vasculogenesis in the embryo). VEGF-C and VEGF-D, but not VEGF-A, are ligands for a third receptor (VEGFR-3), which mediates lymphangiogenesis.

Production

$VEGF_{xxx}$ production can be induced in cells that are not receiving enough oxygen. When a cell is deficient in oxygen, it produces HIF, hypoxia-inducible factor, a transcription factor. HIF stimulates the release of $VEGF_{xxx}$, among other functions (including modulation of erythropoeisis). Circulating $VEGF_{xxx}$ then binds to VEGF Receptors on endothelial cells, triggering a Tyrosine Kinase Pathway leading to angiogenesis.

HIF1 alpha and HIF1 beta are constantly being produced but HIF1 alpha is highly O2 labile, so, in aerobic conditions, it is degraded. When the cell becomes hypoxic, HIF1 alpha persists and the HIF1alpha/beta complex stimulates VEGF release.

Clinical significance

$VEGF_{xxx}$ has been implicated with poor prognosis in breast cancer. Numerous studies show a decreased overall survival and disease-free survival in those tumors overexpressing VEGF. The overexpression of $VEGF_{xxx}$ may be an early step in the process of metastasis, a step that is involved in the "angiogenic" switch. Although $VEGF_{xxx}$ has been correlated with poor survival, its exact mechanism of action in the progression of tumors remains unclear.

$VEGF_{xxx}$ is also released in rheumatoid arthritis in response to TNF-α, increasing endothelial permeability and swelling and also stimulating angiogenesis (formation of capillaries).

$VEGF_{xxx}$ is also important in diabetic retinopathy (DR). The microcirculatory problems in the retina of people with diabetes can cause retinal ischaemia, which results in the release of $VEGF_{xxx}$, and a switch in the balance of pro-angiogenic $VEGF_{xxx}$ isoforms over the normally expressed $VEGF_{xxx}b$ isoforms. $VEGF_{xxx}$ may then cause the creation of new blood vessels in the retina and elsewhere in the eye, heralding changes that may threaten the sight.

$VEGF_{xxx}$ plays a role in the disease pathology of the wet form age-related macular degeneration (AMD), which is the leading cause of blindness for the elderly of the industrialized world. The vascular pathology of AMD shares certain similarities with diabetic retinopathy, although the cause of disease and the typical source of neovascularization differes between the two diseases.

VEGF-D serum levels are significantly elevated in patients with angiosarcoma.

Once released, $VEGF_{xxx}$ may elicit several responses. It may cause a cell to survive, move, or further differentiate. Hence, VEGF is a potential target for the treatment of cancer. The first anti-VEGF drug, a monoclonal antibody named bevacizumab, was approved in 2004. Approximately 10-15% of patients benefit from bevacizumab therapy; however, biomarkers for bevacizumab efficacy are not yet known.

Current studies show that VEGFs are not the only promoters of angiogenesis. In particular FGF2 and HGF are potent angiogenic factors.

Patients suffering from pulmonary emphysema have been found to have decreased levels of VEGF in the pulmonary arteries.

In the kidney, increased expression of $VEGF_{xxx}$ in glomeruli directly causes the glomerular hypertrophy that is associated with proteinuria.

Anti-VEGF therapies

Anti-VEGF therapies are important in the treatment of certain cancers and in age-related macular degeneration. They can involve monoclonal antibodies such as bevacizumab (Avastin), antibody derivatives such as ranibizumab (Lucentis), or orally-available small molecules that inhibit the tyrosine kinases stimulated by VEGF: lapatinib (Tykerb), sunitinib (Sutent), sorafenib (Nexavar), axitinib, and pazopanib.

Both antibody-based compounds are commercialized. The first three orally available compounds are commercialized, as well. The latter two are in clinical trials, the results of which were presented (June 7) at the American Society of Clinical Oncology meeting.

Bergers and Hanahan concluded in 2008 that anti-VEGF drugs can show therapeutic efficacy in mouse models of cancer and in an increasing number of human cancers. But, "the benefits are at best transitory and are followed by a restoration of tumour growth and progression."

AZ2171, a multi-targeted tyrosine kinase inhibitor has been shown to have antiedema effects by reducing the permeability and aiding in vascular normalization.

VEGF is also inhibited by thiazolidinediones (used for diabetes mellitus type 2 and related disease), and this effect on granulosa cells gives the potential of thiazolidinediones to be used in ovarian hyperstimulation syndrome.

Ranibizumab vs. Bevacizumab for Treating Neovascular Age-related Macular Degeneration

Ranibizumab, a monoclonal antibody fragment (Fab) derived from bevacizumab, has been developed by Genetech for intraocular use. In 2004, FDA approved the drug for oncologic use to treat neovascular age-related macular degeneration (wet AMD). The drug has undergone extensive clinical trials.

In the October 2006 issue of the New England Journal of Medicine (NEJM), Rosenfield, et al. reported that monthly intravitreal injection of ranibizumab led to significant increase in the level of mean visual acuity compared to that of sham injection. It was concluded from the two year, phase III study that ranibizumab is very effective in the treatment of minimally classic (MC) or occult wet AMD with low rates of ocular adverse effects.

Another study published in the January 2009 issue of Ophthalmology provides the evidence for the efficacy of ranibizumab. Brown, et al. reported that monthly intravitreal injection of ranibizumab led to significant increase in the level of mean visual acuity compared to that of photodynamic therapy with verteporfin. It was concluded from the two year, phase III study that ranibizumab was superior to photodynamic therapy with verteporfin in the treatment of predominantly classic (PC) Wet AMD with low rates of ocular adverse effects.

Although the efficacy of ranibizumab is well supported by extensive clinical trials, the cost effectiveness of the drug is questioned. Since the drug merely stabilizes patient conditions, ranibizumab must be administered monthly. At a cost of $2,000.00 per injection, the cost to treat wet AMD patients in the United States is greater than $10.00 billion per year. Due to high cost, many ophthalmologists have turned to bevacizumab as the alternative intravitreal agent in the treatment of wet AMD. The drug costs $15.00 to 50.00 in the United States.

In 2007, Raftery, et al. reported in the British Journal of Ophthalmology that, unless ranibizumab is 2.5 times more effective the bevacizumab, ranibizumab is not cost effective. It was concluded that the price of ranibizumab would have to be drastically reduced for the drug to be cost effective.

Off-label use of intravitreal bevacizumab has become a widespread treatment for neovascular age-related macular degeneration. Although the drug is not FDA approved for oncologic uses, some studies suggest that bevacizumab is effective in increasing visual acuity with low rates of ocular adverse effects. However, due to small sample size and lack of randomized control trial, the result is not conclusive.

In October 2006, the National Eye Institute (NEI) of the National Institutes of Health (NIH) announced that it would fund a comparative study trial of ranibizumab and bevacizumab to assess the relative efficacy and ocular adversity in treating wet AMD. This study, called the Comparison of Age-Related Macular Degeneration Treatment

Trials (CATT Study), will enroll about 1,200 patients with newly diagnosed wet AMD, randomly assigning the patients to different treatment groups.

Chapter 7

Platelet-Derived Growth Factor and Fibroblast Growth Factor

Platelet-derived growth factor

Platelet-derived growth factor (PDGF)

Identifiers	
Symbol	PDGF
Pfam	PF00341
InterPro	IPR000072
PROSITE	PDOC00222
SCOP	1pdg

In molecular biology, **platelet-derived growth factor** (**PDGF**) is one of the numerous growth factors, or proteins that regulate cell growth and division. In particular, it plays a significant role in blood vessel formation (angiogenesis), the growth of blood vessels from already-existing blood vessel tissue. Uncontrolled angiogenesis is a characteristic of cancer. In chemical terms, platelet-derived growth factor is dimeric glycoprotein composed of two A (-AA) or two B (-BB) chains or a combination of the two (-AB).

PDGF is a potent mitogen for cells of mesenchymal origin, including smooth muscle cells and glial cells. In both mouse and human, the PDGF signalling network consists of four ligands, PDGFA-D, and two receptors, PDGFRalpha and PDGFRbeta. All PDGFs function as secreted, disulphide-linked homodimers, but only PDGFA and B can form functional heterodimers.

Types/Classification

There are five different isoforms of PDGF that activate cellular response through two different receptors. Known ligands include A (*PDGFA*), B (*PDGFB*), C (*PDGFC*), and D (*PDGFD*), and an AB heterodimer and receptors alpha (*PDGFRA*) and beta (*PDGFRB*). PDGF has few other members of the family, for example VEGF sub-family.

Mechanisms

The receptor for PDGF, **PDGFR** is classified as a receptor tyrosine kinase (RTK), a type of cell surface receptor. Two types of PDGFRs have been identified: alpha-type and beta-type PDGFRs. The alpha type binds to PDGF-AA, PDGF-BB and PDGF-AB, whereas the beta type PDGFR binds with high affinity to PDGF-BB and PDGF-AB. PDGF binds to PDGFRs ligand binding pocket located within the second and third immunoglobulin domains. Upon activation by PDGF, these receptors dimerise, and are "switched on" by auto-phosphorylation of several sites on their cytosolic domains, which serve to mediate binding of cofactors and subsequently activate signal transduction, for example, through the PI3K pathway. Downstream effects of this include regulation of gene expression and the cell cycle. The role of PI3K has been investigated by several laboratories. Accumulating data suggests that, while this molecule is, in general, part of growth signaling complex, it plays a more profound role in controlling cell migration. The different ligand isoforms have variable affinities for the receptor isoforms, and the receptor isoforms may variably form hetero- or homo- dimers. This leads to specificity of downstream signaling. It has been shown that the *cis* oncogene is derived from the PDGF B-chain gene. PDGF-BB is the highest-affinity ligand for the PDGFR-beta; PDGFR-beta is a key marker of hepatic stellate cell activation in the process of fibrogenesis.

Function

PDGFs are mitogenic during early developmental stages, driving the proliferation of undifferentiated mesenchyme and some progenitor populations. During later maturation stages, PDGF signalling has been implicated in tissue remodelling and cellular differentiation, and in inductive events involved in patterning and morphogenesis. In addition to driving mesenchymal proliferation, PDGFs have been shown to direct the migration, differentiation and function of a variety of specialised mesenchymal and migratory cell types, both during development and in the adult animal. Other growth factors in this family include vascular endothelial growth factors B and C (VEGF-B, VEGF-C) which are active in angiogenesis and endothelial cell growth, and placenta growth factor (PlGF) which is also active in angiogenesis.

PDGF plays a role in embryonic development, cell proliferation, cell migration, and angiogenesis. PDGF has also been linked to several diseases such as atherosclerosis, fibrosis and malignant diseases.

In addition, PDGF is a required element in cellular division for fibroblast, a type of connective tissue cell. In essence, the PDGFs allow a cell to skip the G1 checkpoints in order to divide.

PDGF is also known to maintain proliferation of oligodendrocyte progenitor cells.

History

PDGF was one of the first growth factors characterized , and has led to an understanding of the mechanism of many growth factor signaling pathways.

Clinical significance

Like many other growth factors that have been linked to disease, PDGF and its receptors have provided a market for receptor antagonists to treat disease. Such antagonists include (but are not limited to) specific antibodies that target the molecule of interest, which act only in a neutralizing manner.

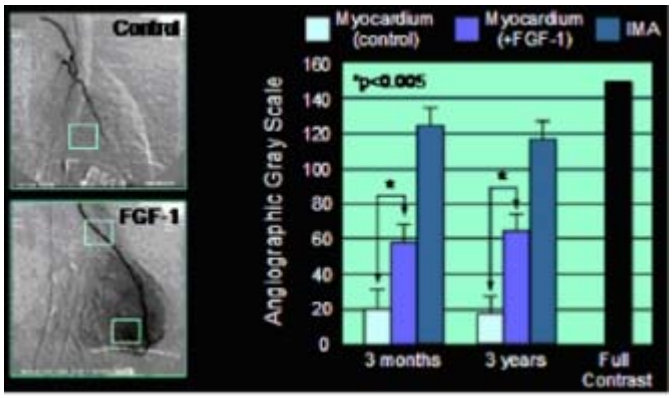
The "c-Sis" oncogene is derived from PDGF.

Family members

Human genes encoding proteins that belong to the platelet-derived growth factor family include:

- FIGF
- PDGFA; PDGFB; PDGFC; PDGFD
- PGF
- VEGF; VEGF41; VEGFB; VEGFC;

Fibroblast growth factor



Angiogenesis

Fibroblast growth factors, or **FGFs**, are a family of growth factors involved in angiogenesis, wound healing, and embryonic development. The FGFs are heparin-binding proteins and interactions with cell-surface associated heparan sulfate proteoglycans have been shown to be essential for FGF signal transduction. FGFs are key players in the processes of proliferation and differentiation of wide variety of cells and tissues.

Families

In humans, 22 members of the FGF family have been identified, all of which are *structurally* related signaling molecules:

- Members FGF1 through FGF10 all bind fibroblast growth factor receptors (FGFRs). FGF1 is also known as *acidic*, and FGF2 is also known as *basic fibroblast growth factor*.
- Members FGF11, FGF12, FGF13, and FGF14, also known as FGF homologous factors 1-4 (FHF1-FHF4), have been shown to have distinct *functional* differences compared to the FGFs. Although these factors possess remarkably similar sequence homology, they do not bind FGFRs and are involved in intracellular processes unrelated to the FGFs. This group is also known as "iFGF".
- Members FGF16 through FGF23 are newer and not as well characterized. FGF15 is the mouse ortholog of human FGF19 (hence there is no human FGF15).
- Human FGF20 was identified based on its homology to *Xenopus* FGF-20 (XFGF-20).
- In contrast to the local activity of the other FGFs, FGF15/FGF19, FGF21 and FGF23 have more systemic effects.

Receptors

The mammalian fibroblast growth factor receptor family has 4 members, FGFR1, FGFR2, FGFR3, and FGFR4. The FGFRs consist of three extracellular immunoglobulin-type domains (D1-D3), a single-span trans-membrane domain and an intracellular split tyrosine kinase domain. FGFs interact with the D2 and D3 domains, with the D3 interactions primarily responsible for ligand-binding specificity. Heparan sulfate binding is mediated through the D3 domain. A short stretch of acidic amino acids located between the D1 and D2 domains has auto-inhibitory functions. This 'acid box' motif interacts with the heparan sulfate binding site to prevent receptor activation in the absence of FGFs.

Alternate mRNA splicing gives rise to 'b' and 'c' variants of FGFRs 1, 2 and 3. Through this mechanism seven different signaling FGFR sub-types can be expressed at the cell surface. Each FGFR binds to a specific subset of the FGFs. Similarly most FGFs can bind to several different FGFR subtypes. FGF1 is sometimes referred to as the 'universal ligand' as it is capable of activating all 7 different FGFRs. In contrast, FGF7 (keratinocyte growth factor, KGF) binds only to FGFR2b (KGFR).

The signaling complex at the cell surface is believed to be a ternary complex formed between two identical FGF ligands, two identical FGFR subunits and either one or two heparan sulfate chains.

History

Fibroblast growth factor was found in pituitary extracts by Armelin in 1973 and then was also found in a cow brain extract by Gospodarowicz et al. and tested in a bioassay which caused fibroblasts to proliferate (first published report in 1974).

They then further fractionated the extract using acidic and basic pH and isolated two slightly different forms that were named "acidic fibroblast growth factor" (FGF1) and "basic fibroblast growth factor" (FGF2). These proteins had a high degree of amino acid identity but were determined to be distinct mitogens. Human FGF2 occurs in low molecular weight (LMW) and high molecular weight (HMW) isoforms. LMW FGF2 is primarily cytoplasmic and functions in an autocrine manner, whereas HMW FGF2s are nuclear and exert activities through an intracrine mechanism.

Not long after FGF1 and FGF2 were isolated, another group isolated a pair of heparin-binding growth factors which they named HBGF-1 and HBGF-2, whilst a third group isolated a pair of growth factors that caused proliferation of cells in a bioassay containing blood vessel endothelium cells which they called ECGF1 and ECGF2. These proteins were found to be identical to the acidic and basic FGFs described by Gospodarowicz et al.

Function

FGFs are multifunctional proteins with a wide variety of effects; they are most commonly mitogens but also have regulatory, morphological, and endocrine effects. They have been alternately referred to as "pluripotent" growth factors and as "promiscuous" growth factors due to their multiple actions on multiple cell types. Promiscuous refers to the biochemistry and pharmacology concept of how a variety of molecules can bind to and elicit a response from single receptor. In the case of FGF, four receptor subtypes can be activated by more than twenty different FGF ligands. Thus the functions of FGFs in developmental processes include mesoderm induction, antero-posterior patterning, limb development, neural induction and neural development, and in mature tissues/systems angiogenesis, keratinocyte organization, and wound healing processes.

FGF is critical during normal development of both vertebrates and invertebrates and any irregularities in their function leads to a range of developmental defects.

One important function of FGF1 and FGF2 is the promotion of endothelial cell proliferation and the physical organization of endothelial cells into tube-like structures. They thus promote angiogenesis, the growth of new blood vessels from the pre-existing vasculature. FGF1 and FGF2 are more potent angiogenic factors than vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF).

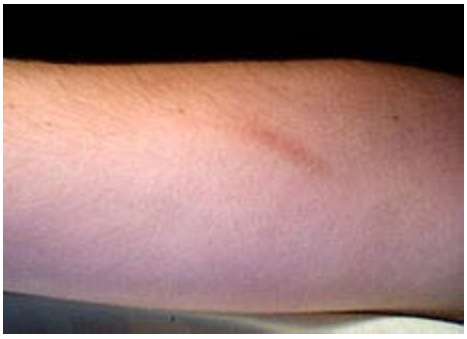
As well as stimulating blood vessel growth, FGFs are important players in wound healing. FGF1 and FGF2 stimulate angiogenesis and the proliferation of fibroblasts that give rise to granulation tissue, which fills up a wound space/cavity early in the wound healing process. FGF7 and FGF10 (also known as Keratinocyte Growth Factors KGF and KGF2, respectively) stimulate the repair of injured skin and mucosal tissues by stimulating the proliferation, migration and differentiation of epithelial cells, and they have direct chemotactic effects on tissue remodeling.

Most FGFs are secreted proteins that bind heparan sulfates and can therefore be caught up in the extracellular matrix of tissues that contain heparan sulfate proteoglycans. This allows them to act locally in a paracrine fashion. However, the FGF19 subfamily (including FGF19, FGF21, and FGF23) which binds less tightly to heparan sulfates can act in an endocrine fashion on far away tissues, such as intestine, liver, kidney, adipose, and bone. For example, FGF19 is produced by intestinal cells but acts on FGFR4-expressing liver cells to downregulate key genes in the bile acid synthase pathway; FGF23 is produced by bone but acts on FGFR1-expressing kidney cells to regulate the synthesis of vitamin D and in turn affect calcium homeostasis.

Chapter 8

Scar

Scar



A minor scar from a cut to the forearm, approx. one year since the wound.

ICD-10	L90.5
MeSH	D002921

Scars (also called **cicatrices**) are areas of fibrous tissue (fibrosis) that replace normal skin (or other tissue) after injury. A scar results from the biologic process of wound repair in the skin and other tissues of the body. Thus, scarring is a natural part of the healing process. With the exception of very minor lesions, every wound (e.g. after accident, disease, or surgery) results in some degree of scarring. An exception to this is animals with regeneration, which do not form scars and the tissue will grow back exactly as before.

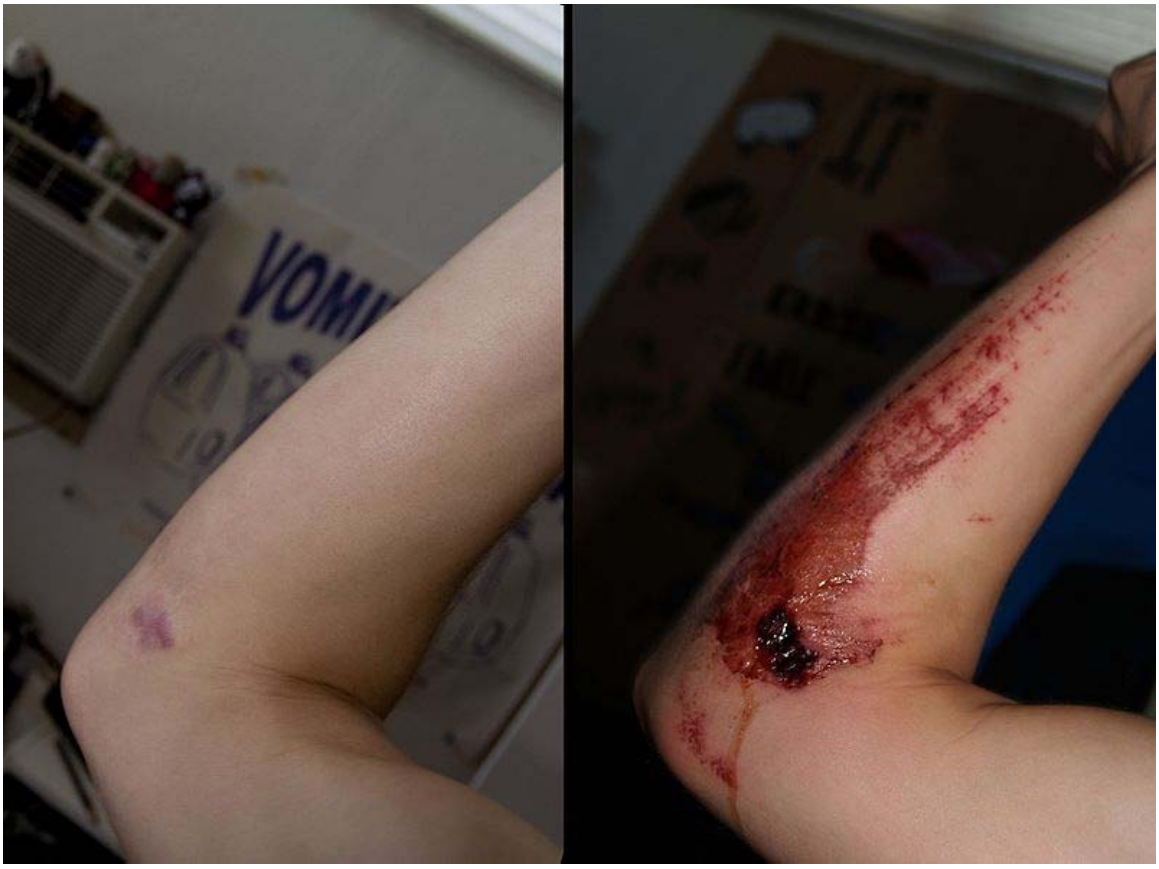
Scar tissue is composed of the same protein (collagen) as the tissue that it replaces, but instead of a random basketweave formation of the collagen fibers found in normal tissue, the collagen cross-links and forms a pronounced alignment in a single direction. This collagen scar tissue alignment is usually of inferior functional quality to the normal collagen randomised alignment. For example, scars in the skin are less resistant to ultraviolet radiation, and sweat glands and hair follicles do not grow back within scar

tissue. A myocardial infarction, commonly known as a heart attack, causes scar formation in the heart muscle, which leads to loss of muscular power and possibly heart failure. However, there are some tissues (e.g. bone) that can heal without any structural or functional deterioration.

Etymology

First attested in English in late 14th century, the word *scar* derives from Old French *escharre*, from Late Latin *eschara*, which is the latinisation of the Greek ἐσχάρα (*eskhara*), meaning "hearth, fire-place", but in medicine "scab, eschar on a wound caused by burning or otherwise".

How scarring occurs



Hypertrophic scarring one year after Road rash on the left, and the original wound on the right.

A scar is a result of the body's patch up reaction after injury on many tissues.

Any injury does not become a scar until the wound has completely healed; this can take many months, or years in the worst pathological cases like keloids. To begin to patch the defect a clot is created; the clot is the beginning process that results in a provisional

matrix. In the process the first layer is a provisional matrix and is not scar. Over time the wounded body tissue then over-expresses collagen inside the provisional matrix to create a collagen matrix. This collagen over expression continues and cross-links the fiber arrangement inside the collagen matrix, making the collagen dense. This densely-packed collagen, morphing into an inelastic whitish collagen scar wall, blocks off cell communication and regeneration and as a result, the new tissue that is generated will have a different texture and quality than the surrounding non-wounded tissue. This prolonged collagen-producing process results in a fortuna scar.

The scarring is created by fibroblast proliferation, a process that begins with a reaction to the clot.

To mend the damage, fibroblasts slowly form the collagen scar. The fibroblast proliferation is circular and cyclically, the fibroblast proliferation lays down thick whitish collagen inside the provisional and collagen matrix, resulting in the packed abundant production of collagen on the fibers giving scars their uneven texture. Over time, the fibroblasts continue to crawl around the matrix, adjusting more fibers and, in the process, the scarring settles and becomes stiff. This fibroblast proliferation also contracts the tissue. In non-wounded tissue, these fibers are not over expressed with thick collagen and do not contract.

As well as the fibroblast proliferation there is prolonged inflammation.

Redness that often follows an injury to the skin is not a scar, and is generally not permanent. The time it takes for this redness to dissipate may, however, range from a few days to, in some serious and rare cases, a few years.

Scars form differently based on the location of the injury on the body and the age of the person who was injured.

The worse the initial damage is, the worse the scar will generally be.

Skin Scars: Skin scars occur when the dermis (the deep, thick layer of skin) is damaged. Most skin scars are flat and leave a trace of the original injury that caused them.

Scar types

The collagen over expression in scarring can be over expressed in different amounts with varying results.

Collagen over expression types include: hypertrophic and keloid scars, both of which experience excessive stiff collagen bundled growth over extending the tissue, blocking off regeneration of tissues. Atrophic scarring (sunken scarring) also has an over expression of collagen blocking of regeneration, this scar type is sunken, the collagen bundles do not over extend the tissue. Stretch marks (striae) are regarded as scars to some.

Hypertrophic

Hypertrophic scars occur when the body overproduces collagen, which causes the scar to be raised above the surrounding skin. Hypertrophic scars take the form of a red raised lump on the skin. Keloid scars are a more serious form of scarring, because they can carry on growing indefinitely into a large, tumorous (although benign) neoplasm.

Hypertrophic scars are often distinguished from keloid scars by their lack of growth outside the original wound area, but this commonly taught distinction can lead to confusion. All keloid scars are hypertrophic but "only a small percentage of large scars" are keloid. Phenotypic differences exist between keloid scars and hypertrophic scars. Keloid scars can occur on anyone, but they are most common in dark-skinned people. Keloid scars can be caused by surgery, an accident, by acne or, sometimes, from body piercings. In some people, keloid scars form spontaneously. Although they can be a cosmetic problem, keloid scars are only inert masses of collagen and therefore completely harmless and non-cancerous. However, they can be itchy or painful in some individuals. They tend to be most common on the shoulders and chest.

Atrophic

An atrophic scar takes the form of a sunken recess in the skin, which has a pitted appearance. These are caused when underlying structures supporting the skin, such as fat or muscle, are lost. This type of scarring is associated with acne, chickenpox, other diseases, surgery or accidents.

Stretch marks

Stretch marks (technically called *striae*) are also a form of scarring. These are caused when the skin is stretched rapidly (for instance during pregnancy, significant weight gain or adolescent growth spurts), or when skin is put under tension during the healing process, (usually near joints). This type of scar usually improves in appearance after a few years.

High corticosteroid levels seam to be contributing if not causative factor in development of striae.

Treatments

According to the American Academy of Dermatology, no scar can be completely removed although in some cases healing can occur without scarring such as healing in embryos, healing without injury (regeneration), and some animals. It also depends on race. Eurasians or asians can have it completely removed and some Africans can. As of 2004 no prescription drugs for the treatment or prevention of scars were available.

Ace Inhibitors

An Ace inhibitor Enalapril at a low dose, over a set period, has been shown to resolve completely hypertrophic scarring and reduce keloid scarring. Another ace inhibitor, a 5% Captopril solution has also been shown to reduce the fibrotic tissue in keloids.

Bark Extract

Basic research in animals suggests that *Spathodea* bark extract, *Centella asiatica* extract, *Anogeissus latifolia* bark extract, and *Channa striata* fish extract in combination with cetrimide cream (a constituent in Savlon) may play a role in wound healing and scar treatment.

Bee Venom

An *alternative* way to remove scars is to dissolve them with enzymes. According to Singh, Ratner etal and Lee, Bee Venom Therapy (BVT) is useful in diminishing scars. They explain when scars are stung they are broken down, softened and faded by substances in the venom. (Bee sting image: Before and After).

Chemical peels

Chemical peels are chemicals which destroy the epidermis in a controlled manner, leading to exfoliation and removing certain skin conditions including superficial scars. Various chemicals can be used depending upon the "depth" of the peel and caution should be used, particularly for dark-skinned individuals and also including individuals susceptible to keloid formation or those with active infections.

Collagen injections

Collagen injections can be used to raise sunken scars to the level of surrounding skin. Its effects can be temporary but will run the chance of a permanent flattening, and it needs to be regularly repeated. There is also a risk in some people of an allergic reaction.

Dermabrasion

Dermabrasion involves the removal of the surface of the skin with specialist equipment or not and usually involves a local anaesthetic.

Laser surgery & resurfacing

The use of lasers on scars is a new form of treatment.

Several cosmetic lasers have been FDA approved for the treatment of acne scars by using laser resurfacing techniques. Vascular lasers have been proven to greatly reduce the redness of most scars 6–10 weeks after the initial treatment.

Carbon dioxide ablative fractional resurfacing is a laser treatment that has been used in acne scarring. It has been theorized that removing layers of skin with a carbon dioxide or Erbium:YAG laser may help flatten scars.

Atrophic Scarring occurring after surgical procedures or trauma is a common cosmetic problem for patients. Atrophic scars, which present as topographical depressions, result when dermal collagen and connective tissue production during the physiologic wound-healing process inadequately compensate for the tissue loss present after injury. Wound tension, tissue apposition, individual variations in wound healing, and scar contraction are all factors that contribute to the creation of a depressed, atrophic scar. With varying success, numerous ablative, nonablative, and fractional devices have been used to stimulate neocollagenesis and dermal remodeling in an attempt to improve the appearance of atrophic scars.

Miscellaneous

In 1971 *Moss & Clifford*, produced a patent that claimed scar free healing. Their work went unnoticed and was not peer reviewed.

Needling

Needling, also called subcision, dermarolling, or percutaneous collagen induction therapy, began in 1997. It is a process where the scarred area is continuously needled to promote collagen formation. In 2008 a retrospective analysis of 480 persons concluded that it was effective; the patients applied vitamin A and vitamin C to the skin prior to and following the needling. A 2009 review of the therapy similarly concluded that it was effective.

The needles are typically standard medical grade stainless steel or newer variants made from titanium which can have minimal diameter yet retain strength and sharpness for reducing pain. The needles are fixed onto a plastic barrel which rotates around an axle that connects to a handle for holding the device. Once needled the area is allowed to fully heal, and needled again if required depending on the intensity of the scar. Scarring needles and needling rollers are available for home use; however, needling should not be done on parts of the face or areas where major nerves are located without professional medical supervision. Needling at home must also be done in line with hygienic and sterilization requirements. Despite the small length of home use needles, it is prudent to ensure that the microneedle roller has been gamma sterilised by the manufacturer as usually these devices are assembled by hand.

It is worth noting that severe scarring is unlikely to benefit from home based treatments as the user is unlikely to be able to penetrate deeply enough to create significant improvements. In the cases of deep scarring, only professional treatments are likely to work.

Other & Experimental

An intradermal injection of Transforming Growth Factor Beta 3 (TGFβ3) is being tested. The results of three trials already completed were published in the *Lancet* along with an editorial commentary.

A study implicated the protein Ribosomal s6 kinase (RSK) in the formation of scar tissue and found that the introduction of a chemical to counteract RSK could halt the formation of Cirrhosis. This treatment also has the potential to reduce or even prevent altogether other types of scarring.

Research has also implicated osteopontin in scarring.

Over the counter topical remedies

Ointments and semiocclusive dressings including mineral oil, lotions, and petrolatum-based ointments are recommended under guidelines as they promote moist healing. One 1996 trial found healing was improved but physical characteristics were unchanged, while a 2007 trial of a triple-antibiotic ointment found reduced scarring.

Radiotherapy

Low-dose, superficial radiotherapy, is used to prevent re-occurrence of severe keloid and hypertrophic scarring. It is usually effective, but only used in extreme cases due to the risk of long-term side effects.

- Is Radiation Therapy for Keloids Acceptable? The Risk of Radiation-Induced Carcinogenesis Article

Semiocclusive Ointments & Pressure dressing

Semiocclusive ointments (e.g. petrolatum-based), silicone gel sheeting and steroid injections have a widely-accepted role in general scar treatment,. In 1962, a paper supporting the use of a semiocclusive ointments to speed healing and reduce scarring was published, beginning a practice which is now "a cornerstone of wound care" and the beginning of the discovery of the effectiveness of occlusive methods (ointments, occlusive dressings, silicones). The effectiveness of silicone gel over nonsilicone gel was initially seen as controversial as no significant differences were noted when comparing silicone vs non silicone dressings. It is now more accepted that the silicone itself is not a biologically active part of scar formation, it is the hydration silicone (and other occlusive dressings) offer. In 2002, Mustoe et al. in Vol 110. No 2 of Plastic and Reconstructive Surgery offer the International Recommendations on Scar Management and state, a "primary role for silicone gel sheeting and (corticosteroid injections) for the management of a wide variety of abnormal scars". Corticosteroid therapy by injection into the scars was also introduced in the 1960s. From the early 1970s pressure garment therapy was introduced for widespread burn scars, and silicone gel sheets from the 1980s.

Pressure dressings are commonly used in managing burn and hypertrophic scars, although supporting evidence is lacking. These involve elastic materials or gauze which apply pressure to the area. For large scars and particularly large burns, pressure garments may be worn. It is believed that they work by applying constant pressure to surface blood vessels and eventually causing scars to flatten and become softer. Retrospective and ultrasonic studies since the 1960s have supported their use, but the only randomized clinical trial found no statistically significant difference in wound healing. Care providers commonly report improvements, however, and pressure therapy has been effective in treating ear keloids. The general acceptance of the treatment as effective may prevent it from being further studied in clinical trials.

Silicone scar treatments improve scar appearance and are often used to prevent and treat hypertrophic scarring. Although clinical studies spanning 20+ years prove it works, the exact mechanism of action is not fully understood . Studies have suggested several modes of action, quite possible a combination of effects including a manipulation of local ionic charges, an increase in the collagenase activity at the site (Collagenases are enzymes that break the peptide bonds in collagen), or a decrease in production of pro-inflammatory substances like TGFβ2.

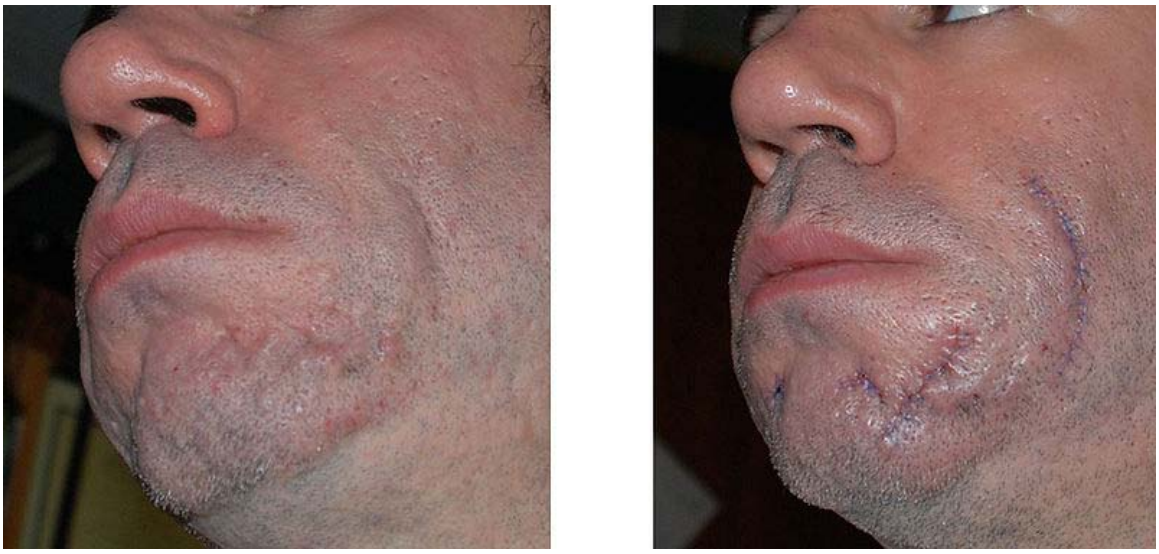
Steroids

A long term course of steroid injections under medical supervision, into the scar may help flatten and soften the appearance of keloid or hypertrophic scars.

The steroid is injected into the scar itself; since very little is absorbed into the blood stream, side effects of this treatment are minor. However, it does cause thinning of the scar tissue so it does carry risks when injected into scars caused by operations into ruptured tendons. This treatment is repeated at 4-6 week intervals.

Topical steroids are ineffective.

Surgery



Scarring caused by acne (left), and photo 1 day after scar revision surgery. Area around sutures is still swollen from surgery

Scar revision is a process of cutting the scar tissue out. After the excision, the new wound is usually closed up in order to heal by primary intention, instead of secondary intention. Deeper cuts need a multi-layered closure to heal optimally, otherwise depressed or dented scars can result.

Surgical excision of hypertrophic or keloid scars is often associated to other methods such as pressotherapy or silicone gel sheeting. Lone excision of keloid scars however shows a high recurrence rate close to 45%. A clinical study is currently ongoing to assess the benefits of a treatment combining surgery and laser-assisted healing in hypertrophic or keloid scars.

Vitamin C

Stable forms of topical vitamin C have been shown to improve collagen formation.

Vitamin E

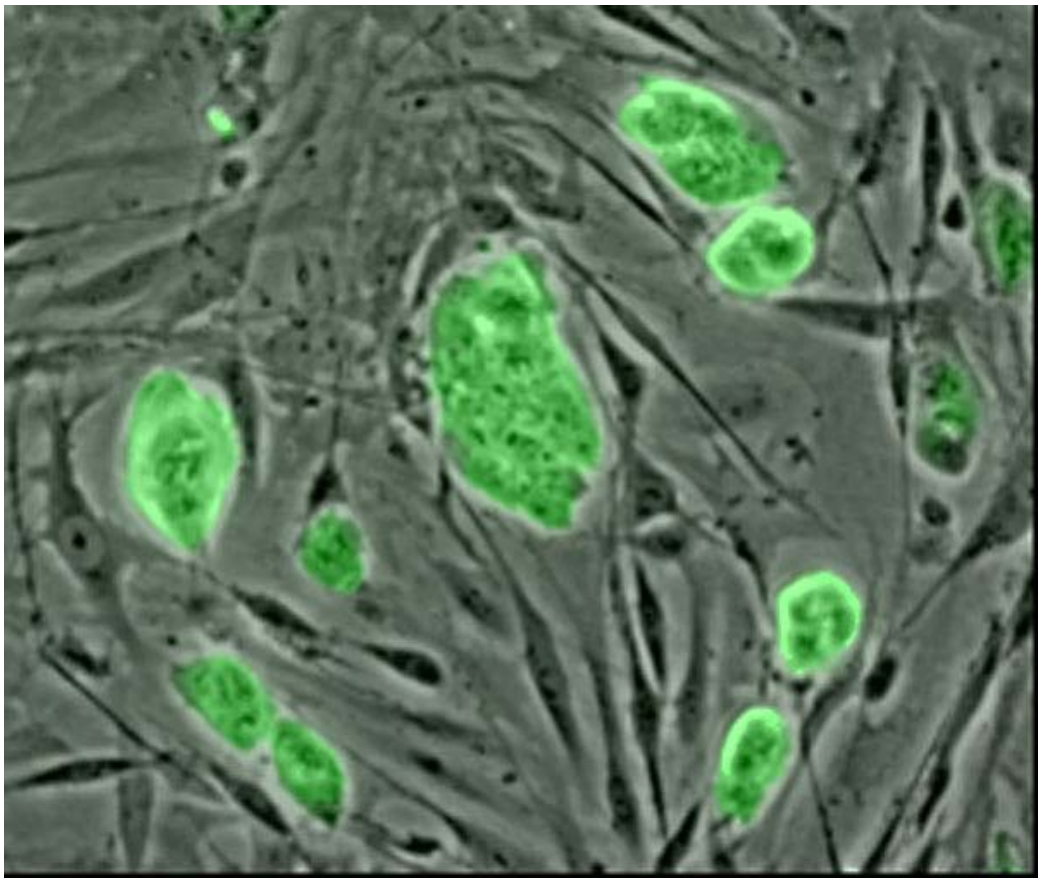
Research shows the use of vitamin E and onion extract (sold under Mederma) as treatments for scars is ineffective. Vitamin E causes contact dermatitis in up to 33% of users and in some cases it may worsen scar appearance. Vitamin C and some of its esters also fade the dark pigment associated with some scars.

Intentional scarring

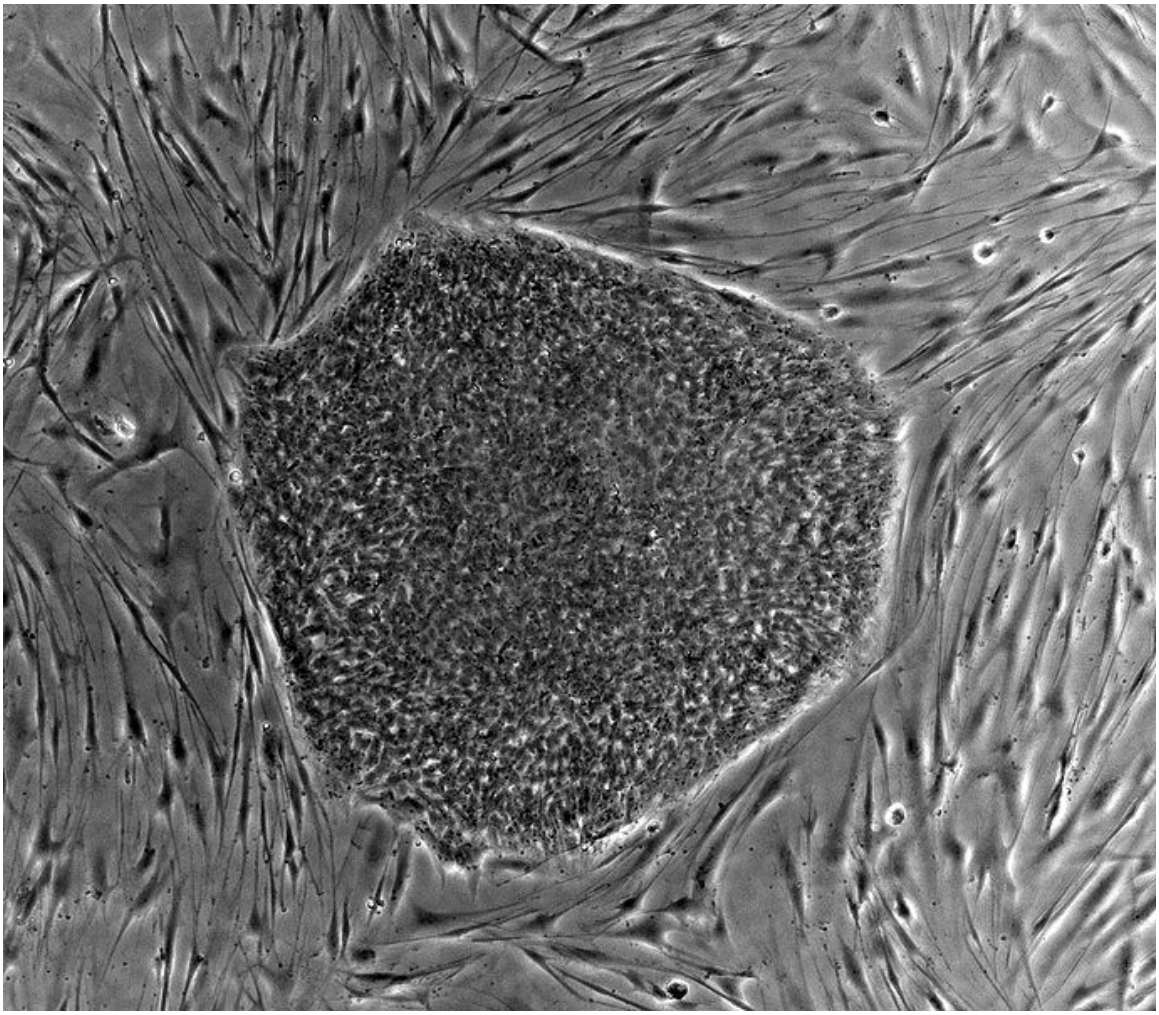
The permanence of scarring has led to its intentional use as a form of body art within some cultures and subcultures. These forms of ritual and non-ritual scarring practices can be found in many groups and cultures around the world.

Chapter 9

Stem Cell



Mouse embryonic stem cells with fluorescent marker



Human embryonic stem cell colony on mouse embryonic fibroblast feeder layer

Stem cells are biological cells found in all multicellular organisms, that can divide through mitosis and differentiate into diverse specialized cell types. In mammals, there are two broad types of stem cells: embryonic stem cells that are isolated from the inner cell mass of blastocysts, and adult stem cells that are found in various tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenished in adult tissues. In a developing embryo, stem cells can differentiate into all the specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.

Stem cells can now be artificially grown and transformed into specialized cell types with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture. Highly plastic adult stem cells are routinely used in medical therapies. Stem cells can be taken from a variety of sources, including umbilical cord blood and bone marrow. Embryonic cell lines and autologous embryonic stem cells generated through therapeutic cloning have also been proposed as promising candidates for future therapies.

Research of stem cells grew out of findings by Ernest A. McCulloch and James E. Till at the University of Toronto in the 1960s.

Properties

The classical definition of a stem cell requires that it possess two properties:

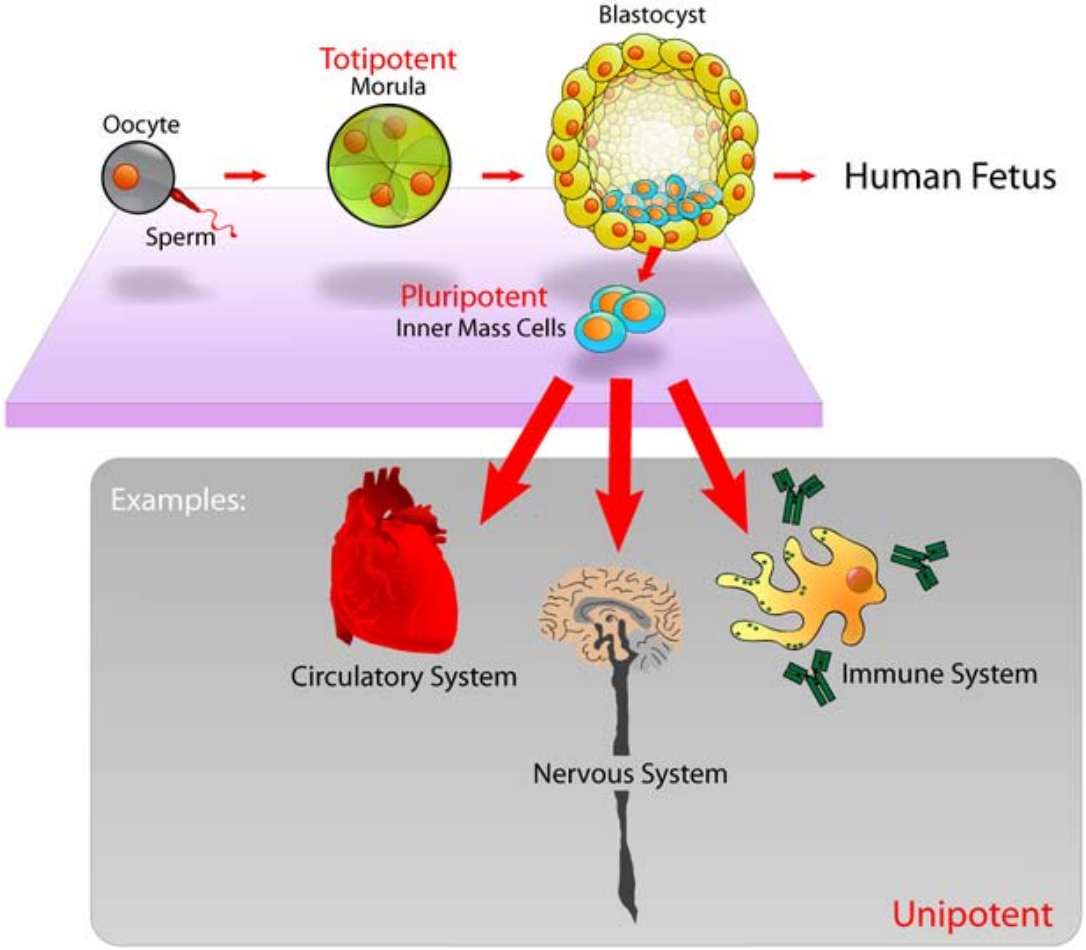
- *Self-renewal* - the ability to go through numerous cycles of cell division while maintaining the undifferentiated state.
- *Potency* - the capacity to differentiate into specialized cell types. In the strictest sense, this requires stem cells to be either totipotent or pluripotent - to be able to give rise to any mature cell type, although multipotent or unipotent progenitor cells are sometimes referred to as stem cells.

Self-renewal

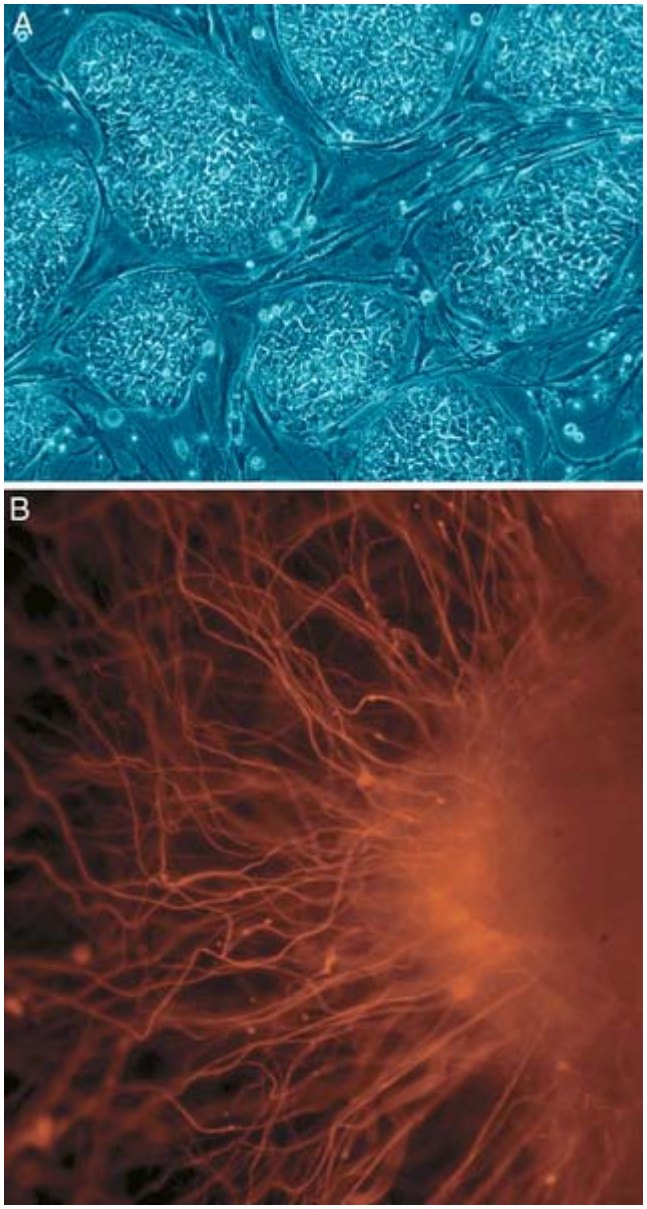
Two mechanisms exist to ensure that the stem cell population is maintained:

1. Obligatory asymmetric replication - a stem cell divides into one father cell that is identical to the original stem cell, and another daughter cell that is differentiated
2. Stochastic differentiation - when one stem cell develops into two differentiated daughter cells, another stem cell undergoes mitosis and produces two stem cells identical to the original.

Potency definitions



Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst. The stem cells can become any tissue in the body, excluding a placenta. Only the morula's cells are totipotent, able to become all tissues and a placenta.



Human embryonic stem cells
A: Cell colonies that are not yet differentiated.
B: Nerve cell

Potency specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cell.

- Totipotent (a.k.a omnipotent) stem cells can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable, organism. These cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent.
- Pluripotent stem cells are the descendants of totipotent cells and can differentiate into nearly all cells, i.e. cells derived from any of the three germ layers.

- Multipotent stem cells can differentiate into a number of cells, but only those of a closely related family of cells.
- Oligopotent stem cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells.
- Unipotent cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells).

Identification

The practical definition of a stem cell is the functional definition - a cell that has the potential to regenerate tissue over a lifetime. For example, the gold standard test for a bone marrow or hematopoietic stem cell (HSC) is the ability to transplant one cell and save an individual without HSCs. In this case, a stem cell must be able to produce new blood cells and immune cells over a long term, demonstrating potency. It should also be possible to isolate stem cells from the transplanted individual, which can themselves be transplanted into another individual without HSCs, demonstrating that the stem cell was able to self-renew.

Properties of stem cells can be illustrated *in vitro*, using methods such as clonogenic assays, where single cells are characterized by their ability to differentiate and self-renew. As well, stem cells can be isolated based on a distinctive set of cell surface markers. However, *in vitro* culture conditions can alter the behavior of cells, making it unclear whether the cells will behave in a similar manner *in vivo*. Considerable debate exists whether some proposed adult cell populations are truly stem cells.

Embryonic

Embryonic stem cell lines (ES cell lines) are cultures of cells derived from the epiblast tissue of the inner cell mass (ICM) of a blastocyst or earlier morula stage embryos. A blastocyst is an early stage embryo—approximately four to five days old in humans and consisting of 50–150 cells. ES cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. In other words, they can develop into each of the more than 200 cell types of the adult body when given sufficient and necessary stimulation for a specific cell type. They do not contribute to the extra-embryonic membranes or the placenta.

Nearly all research to date has taken place using mouse embryonic stem cells (mES) or human embryonic stem cells (hES). Both have the essential stem cell characteristics, yet they require very different environments in order to maintain an undifferentiated state. Mouse ES cells are grown on a layer of gelatin and require the presence of Leukemia Inhibitory Factor (LIF). Human ES cells are grown on a feeder layer of mouse embryonic fibroblasts (MEFs) and require the presence of basic Fibroblast Growth Factor (bFGF or FGF-2). Without optimal culture conditions or genetic manipulation, embryonic stem cells will rapidly differentiate.

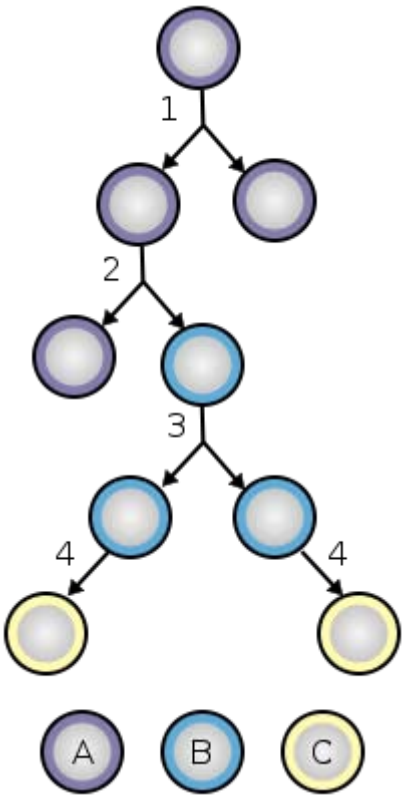
A human embryonic stem cell is also defined by the presence of several transcription factors and cell surface proteins. The transcription factors Oct-4, Nanog, and Sox2 form the core regulatory network that ensures the suppression of genes that lead to differentiation and the maintenance of pluripotency. The cell surface antigens most commonly used to identify hES cells are the glycolipids SSEA3 and SSEA4 and the keratan sulfate antigens Tra-1-60 and Tra-1-81. The molecular definition of a stem cell includes many more proteins and continues to be a topic of research.

After nearly ten years of research, there are no approved treatments using embryonic stem cells. The first human trial was approved by the US Food & Drug Administration in January 2009. However, as of August 2010, the first human trial had not yet been initiated. The first human medical trial for embryonic stem cells started in Atlanta on October 13, 2010 for spinal injury victims. ES cells, being pluripotent cells, require specific signals for correct differentiation - if injected directly into another body, ES cells will differentiate into many different types of cells, causing a teratoma. Differentiating ES cells into usable cells while avoiding transplant rejection are just a few of the hurdles that embryonic stem cell researchers still face. Many nations currently have moratoria on either ES cell research or the production of new ES cell lines. Because of their combined abilities of unlimited expansion and pluripotency, embryonic stem cells remain a theoretically potential source for regenerative medicine and tissue replacement after injury or disease.

Fetal

Fetal stem cells are primitive cell types found in the organs of fetuses.

Adult



Stem cell division and differentiation. A - stem cell; B - progenitor cell; C - differentiated cell; 1 - symmetric stem cell division; 2 - asymmetric stem cell division; 3 - progenitor division; 4 - terminal differentiation

Also known as somatic (from Greek Σωματικός, "of the body") stem cells and germline (giving rise to gametes) stem cells, they can be found in children, as well as adults.

Pluripotent adult stem cells are rare and generally small in number but can be found in a number of tissues including umbilical cord blood. A great deal of adult stem cell research has focused on clarifying their capacity to divide or self-renew indefinitely and their differentiation potential. In mice, pluripotent stem cells are directly generated from adult fibroblast cultures. Unfortunately, many mice don't live long with stem cell organs.

Most adult stem cells are lineage-restricted (multipotent) and are generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, etc.).

Adult stem cell treatments have been successfully used for many years to treat leukemia and related bone/blood cancers through bone marrow transplants. Adult stem cells are also used in veterinary medicine to treat tendon and ligament injuries in horses.

The use of adult stem cells in research and therapy is not as controversial as embryonic stem cells, because the production of adult stem cells does not require the destruction of an embryo. Additionally, because in some instances adult stem cells can be obtained from the intended recipient, (an autograft) the risk of rejection is essentially non-existent in these situations. Consequently, more US government funding is being provided for adult stem cell research.

An extremely rich source for adult mesenchymal stem cells is the developing tooth bud of the mandibular third molar. While considered multipotent they may prove to be pluripotent. The stem cells eventually form enamel (ectodrm), dentin,periodontal ligament, blood vessels, dental pulp, nervous tissues, including a minimum of 29 different unique end organs. Because of extreme ease in collection at 8–10 years of age before calcification and minimal to no morbidity will probably constitute a major source for personal banking, research and multiple therapies. These stem cells have been shown capable of producing hepatocytes.

Amniotic

Multipotent stem cells are also found in amniotic fluid. These stem cells are very active, expand extensively without feeders and are not tumorigenic. Amniotic stem cells are multipotent and can differentiate in cells of adipogenic, osteogenic, myogenic, endothelial, hepatic and also neuronal lines. All over the world, universities and research institutes are studying amniotic fluid to discover all the qualities of amniotic stem cells, and scientists such as Anthony Atala and Giuseppe Simoni have discovered important results.

From an ethical point of view, stem cells from amniotic fluid can solve a lot of problems, because it's possible to catch amniotic stem cells without destroying embryos. For example, the Vatican newspaper "Osservatore Romano" called amniotic stem cell "the future of medicine".

It's possible to collect amniotic stem cells for donors or for autologuous use: the first US amniotic stem cells bank opened in 2009 in Medford, MA, by Biocell Center Corporation and collaborates with various hospitals and universities all over the world.

Induced pluripotent

These are not adult stem cells, but rather reprogrammed cells (e.g. epithelial cells) given pluripotent capabilities. Using genetic reprogramming with protein transcription factors, pluripotent stem cells equivalent to embryonic stem cells have been derived from human adult skin tissue. Shinya Yamanaka and his colleagues at Kyoto University used the transcription factors Oct3/4, Sox2, c-Myc, and Klf4 in their experiments on cells from human faces. Junying Yu, James Thomson, and their colleagues at the University of Wisconsin–Madison used a different set of factors, Oct4, Sox2, Nanog and Lin28, and carried out their experiments using cells from human foreskin.

As a result of the success of these experiments, Ian Wilmut, who helped create the first cloned animal Dolly the Sheep, has announced that he will abandon nuclear transfer as an avenue of research.

Frozen blood samples can be used as a source of induced pluripotent stem cells, opening a new avenue for obtaining the valued cells.

Lineage

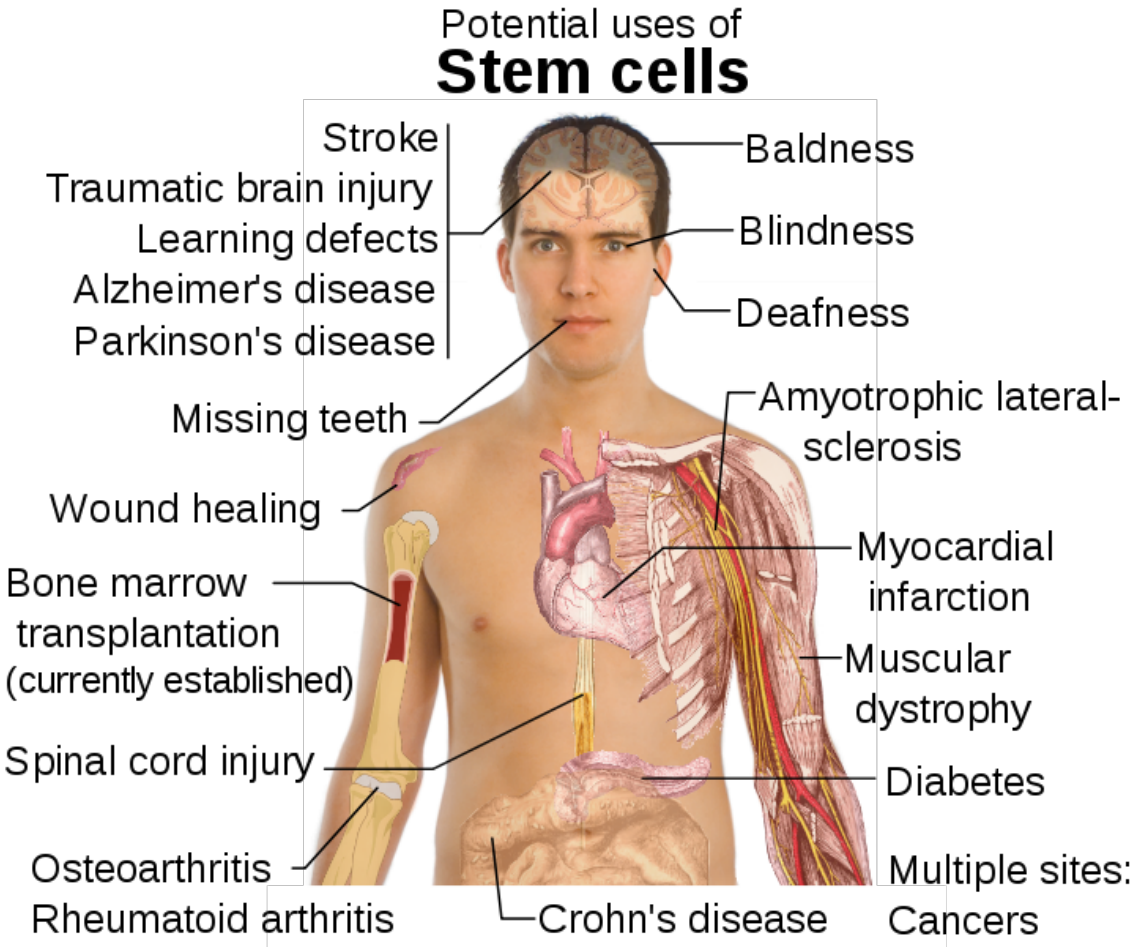
To ensure self-renewal, stem cells undergo two types of cell division. Symmetric division gives rise to two identical daughter cells both endowed with stem cell properties. Asymmetric division, on the other hand, produces only one stem cell and a progenitor cell with limited self-renewal potential. Progenitors can go through several rounds of cell division before terminally differentiating into a mature cell. It is possible that the molecular distinction between symmetric and asymmetric divisions lies in differential segregation of cell membrane proteins (such as receptors) between the daughter cells.

An alternative theory is that stem cells remain undifferentiated due to environmental cues in their particular niche. Stem cells differentiate when they leave that niche or no longer receive those signals. Studies in *Drosophila* germarium have identified the signals dpp and adherens junctions that prevent germarium stem cells from differentiating.

The signals that lead to reprogramming of cells to an embryonic-like state are also being investigated. These signal pathways include several transcription factors including the oncogene c-Myc. Initial studies indicate that transformation of mice cells with a combination of these anti-differentiation signals can reverse differentiation and may allow adult cells to become pluripotent. However, the need to transform these cells with an oncogene may prevent the use of this approach in therapy.

Challenging the terminal nature of cellular differentiation and the integrity of lineage commitment, it was recently determined that the somatic expression of combined transcription factors can directly induce other defined somatic cell fates; researchers identified three neural-lineage-specific transcription factors that could directly convert mouse fibroblasts (skin cells) into fully functional neurons. This "induced neurons" (iN) cell research inspires the researchers to induce other cell types implies that *all* cells are totipotent: with the proper tools, all cells may form all kinds of tissue.

Treatments



Diseases and conditions where stem cell treatment is promising or emerging. Bone marrow transplantation is, as of 2009, the only established use of stem cells.

Medical researchers believe that stem cell therapy has the potential to dramatically change the treatment of human disease. A number of adult stem cell therapies already exist, particularly bone marrow transplants that are used to treat leukemia. In the future, medical researchers anticipate being able to use technologies derived from stem cell research to treat a wider variety of diseases including cancer, Parkinson's disease, spinal cord injuries, Amyotrophic lateral sclerosis, multiple sclerosis, and muscle damage, amongst a number of other impairments and conditions. However, there still exists a great deal of social and scientific uncertainty surrounding stem cell research, which could possibly be overcome through public debate and future research, and further education of the public.

One concern of treatment is the possible risk that transplanted stem cells could form tumors and have the possibility of becoming cancerous if cell division continues uncontrollably.

Stem cells, however, are already studied extensively. While some scientists are hesitant to associate the therapeutic potential of stem cells as the first goal of the research, they find the investigation of stem cells as a goal worthy in itself.

Contrarily, supporters of embryonic stem cell research argue that such research should be pursued because the resultant treatments could have significant medical potential. It is also noted that excess embryos created for in vitro fertilization could be donated with consent and used for the research.

The recent development of iPS cells has been called a bypass of the legal controversy. Laws limiting the destruction of human embryos have been credited for being the reason for development of iPS cells, but it is still not completely clear whether hiPS cells are equivalent to hES cells. Recent work demonstrates hotspots of aberrant epigenomic reprogramming in hiPS cells (Lister, R., et al., 2011).

Research patents

The patents covering a lot of work on human embryonic stem cells are owned by the Wisconsin Alumni Research Foundation (WARF). WARF does not charge academics to study human stem cells but does charge commercial users. WARF sold Geron Corp. exclusive rights to work on human stem cells but later sued Geron Corp. to recover some of the previously sold rights. The two sides agreed that Geron Corp. would keep the rights to only three cell types. In 2001, WARF came under public pressure to widen access to human stem-cell technology.

A request for reviewing the WARF patents 5,843,780; 6,200,806; 7,029,913 US Patent and Trademark Office were filed by non-profit patent-watchdogs The Foundation for Taxpayer & Consumer Rights, and the Public Patent Foundation as well as molecular biologist Jeanne Loring of the Burnham Institute. According to them, two of the patents granted to WARF are invalid because they cover a technique published in 1993 for which a patent had already been granted to an Australian researcher. Another part of the challenge states that these techniques, developed by James A. Thomson, are rendered obvious by a 1990 paper and two textbooks. Based on this challenge, patent 7,029,913 has been rejected in 2010. The two remaining hES WARF patents are due to expire in 2015.

The outcome of this legal challenge is particularly relevant to the Geron Corp. as it can only license patents that are upheld.

Key research events

- 1908 - The term "stem cell" was proposed for scientific use by the Russian histologist Alexander Maksimov (1874–1928) at congress of hematologic society in Berlin. It postulated existence of haematopoietic stem cells.

- 1960s - Joseph Altman and Gopal Das present scientific evidence of adult neurogenesis, ongoing stem cell activity in the brain; like André Gernez, their reports contradict Cajal's "no new neurons" dogma and are largely ignored.
- 1963 - McCulloch and Till illustrate the presence of self-renewing cells in mouse bone marrow.
- 1968 - Bone marrow transplant between two siblings successfully treats SCID.
- 1978 - Haematopoietic stem cells are discovered in human cord blood.
- 1981 - Mouse embryonic stem cells are derived from the inner cell mass by scientists Martin Evans, Matthew Kaufman, and Gail R. Martin. Gail Martin is attributed for coining the term "Embryonic Stem Cell".
- 1992 - Neural stem cells are cultured *in vitro* as neurospheres.
- 1997 - Leukemia is shown to originate from a haematopoietic stem cell, the first direct evidence for cancer stem cells.
- 1998 - James Thomson and coworkers derive the first human embryonic stem cell line at the University of Wisconsin–Madison.
- 1998 - John Gearhart (Johns Hopkins University) extracted germ cells from fetal gonadal tissue (primordial germ cells) before developing pluripotent stem cell lines from the original extract.
- 2000s - Several reports of adult stem cell plasticity are published.
- 2001 - Scientists at Advanced Cell Technology clone first early (four- to six-cell stage) human embryos for the purpose of generating embryonic stem cells.
- 2003 - Dr. Songtao Shi of NIH discovers new source of adult stem cells in children's primary teeth.
- 2004–2005 - Korean researcher Hwang Woo-Suk claims to have created several human embryonic stem cell lines from unfertilised human oocytes. The lines were later shown to be fabricated.
- 2005 - Researchers at Kingston University in England claim to have discovered a third category of stem cell, dubbed cord-blood-derived embryonic-like stem cells (CBEs), derived from umbilical cord blood. The group claims these cells are able to differentiate into more types of tissue than adult stem cells.
- 2005 - Researchers at UC Irvine's Reeve-Irvine Research Center are able to partially restore the ability of mice with paralyzed spines to walk through the injection of human neural stem cells.
- August 2006 - Mouse Induced pluripotent stem cells: the journal *Cell* publishes Kazutoshi Takahashi and Shinya Yamanaka.
- October 2006 - Scientists at Newcastle University in England create the first ever artificial liver cells using umbilical cord blood stem cells.
- January 2007 - Scientists at Wake Forest University led by Dr. Anthony Atala and Harvard University report discovery of a new type of stem cell in amniotic fluid. This may potentially provide an alternative to embryonic stem cells for use in research and therapy.
- June 2007 - Research reported by three different groups shows that normal skin cells can be reprogrammed to an embryonic state in mice. In the same month, scientist Shoukhrat Mitalipov reports the first successful creation of a primate stem cell line through somatic cell nuclear transfer

- October 2007 - Mario Capecchi, Martin Evans, and Oliver Smithies win the 2007 Nobel Prize for Physiology or Medicine for their work on embryonic stem cells from mice using gene targeting strategies producing genetically engineered mice (known as knockout mice) for gene research.
- November 2007 - Human induced pluripotent stem cells: Two similar papers released by their respective journals prior to formal publication: in *Cell* by Kazutoshi Takahashi and Shinya Yamanaka, "Induction of pluripotent stem cells from adult human fibroblasts by defined factors", and in *Science* by Junying Yu, et al., from the research group of James Thomson, "Induced pluripotent stem cell lines derived from human somatic cells": pluripotent stem cells generated from mature human fibroblasts. It is possible now to produce a stem cell from almost any other human cell instead of using embryos as needed previously, albeit the risk of tumorigenesis due to c-myc and retroviral gene transfer remains to be determined.
- January 2008 - Robert Lanza and colleagues at Advanced Cell Technology and UCSF create the first human embryonic stem cells without destruction of the embryo
- January 2008 - Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts
- February 2008 - Generation of pluripotent stem cells from adult mouse liver and stomach: these iPS cells seem to be more similar to embryonic stem cells than the previously developed iPS cells and not tumorigenic, moreover genes that are required for iPS cells do not need to be inserted into specific sites, which encourages the development of non-viral reprogramming techniques.
- March 2008-The first published study of successful cartilage regeneration in the human knee using autologous adult mesenchymal stem cells is published by clinicians from Regenerative Sciences
- October 2008 - Sabine Conrad and colleagues at Tübingen, Germany generate pluripotent stem cells from spermatogonial cells of adult human testis by culturing the cells in vitro under leukemia inhibitory factor (LIF) supplementation.
- 30 October 2008 - Embryonic-like stem cells from a single human hair.
- 1 March 2009 - Andras Nagy, Keisuke Kaji, *et al.* discover a way to produce embryonic-like stem cells from normal adult cells by using a novel "wrapping" procedure to deliver specific genes to adult cells to reprogram them into stem cells without the risks of using a virus to make the change. The use of electroporation is said to allow for the temporary insertion of genes into the cell.
- 28 May 2009 Kim *et al.* announced that they had devised a way to manipulate skin cells to create patient specific "induced pluripotent stem cells" (iPS), claiming it to be the 'ultimate stem cell solution'.
- 11 October 2010 First trial of embryonic stem cells in humans.
- 25 October 2010 - Ishikawa et *al.* write in the Journal of Experimental Medicine that research shows that transplanted cells which contain their new host's nuclear DNA could still be rejected by the invidual's immune system due to foreign mitochondrial DNA. Tissues made from a person's stem cells could therefore be rejected, because mitochondrial genomes tend to accumulate mutations.

Chapter 10

Maggot Therapy

A wound cleaned by maggots

Maggot therapy (also known as maggot debridement therapy (MDT), larval therapy, larva therapy, larvae therapy, biodebridement or biosurgery) is a type of biotherapy involving the intentional introduction of live, disinfected maggots (fly larvae) into the non-healing skin and soft tissue wound(s) of a human or animal for the purposes of selectively cleaning out only the necrotic tissue within a wound (debridement), disinfection, and promotion of wound healing.

History

Early history

Written records have documented that maggots have been used since antiquity as a wound treatment. There are reports of the successful use of maggots for wound healing by Maya Indians and Aboriginal tribes in Australia. There also have been reports of the use of maggot treatment in Renaissance times. During warfare, many military physicians observed that soldiers whose wounds had become colonized with maggots experienced significantly less morbidity and mortality than soldiers whose wounds had not become

colonized. These physicians included Napoleon’s general surgeon, Baron Dominique Larrey, who reported during France's Egyptian campaign in Syria, 1798–1801, that certain species of fly destroyed only dead tissue and had a positive effect on wound healing.

Dr. Joseph Jones, a ranking Confederate medical officer during the American Civil War, is quoted as follows, "I have frequently seen neglected wounds ... filled with maggots ... as far as my experience extends, these worms only destroy dead tissues, and do not injure specifically the well parts." The first therapeutic use of maggots is credited to a second Confederate medical officer Dr. J.F. Zacharias, who reported during the American Civil War that, "Maggots ... in a single day would clean a wound much better than any agents we had at our command ... I am sure I saved many lives by their use." He recorded a high survival rate in patients he treated with maggots.

During World War I, Dr. William S. Baer, an orthopedic surgeon, recognized on the battlefield the efficacy of maggot colonization for healing wounds. He observed one soldier left for several days on the battlefield who had sustained compound fractures of the femur and large flesh wounds of the abdomen and scrotum. When the soldier arrived at the hospital, he had no signs of fever despite the serious nature of his injuries and his prolonged exposure to the elements without food or water. When his clothes were removed, it was seen that "thousands and thousands of maggots filled the entire wounded area." To Dr. Baer's surprise, when these maggots were removed "there was practically no bare bone to be seen and the internal structure of the wounded bone as well as the surrounding parts was entirely covered with most beautiful pink tissue that one could imagine." This case took place at a time when the death rate for compound fractures of the femur was about 75-80%.

Modern use

While at Johns Hopkins University in 1929, Dr. Baer introduced maggots into 21 patients with intractable chronic osteomyelitis. He observed rapid debridement, reductions in the number of pathogenic organisms, reduced odor levels, alkalinization of wound beds, and ideal rates of healing. All 21 patients' open lesions were completely healed and they were released from the hospital after two months of maggot therapy.

After the publication of Dr. Baer's results in 1931, maggot therapy for wound care became very common, particularly in the United States. The Lederle pharmaceutical company commercially produced "Surgical Maggots", larvae of the green bottle fly, which primarily feed on the necrotic tissue of the living host without attacking living tissue. Between 1930 and 1940, more than 100 medical papers were published on maggot therapy. Medical literature of this time contains many references to the successful use of maggots in chronic or infected wounds including osteomyelitis, abscesses, burns, sub-acute mastoiditis, and chronic empyema.

More than 300 American hospitals employed maggot therapy during the 1940s. The extensive use of maggot therapy prior to World War II was curtailed when the discovery

and growing use of penicillin caused it to be deemed outdated. Due to the lack of conventional medicines, maggot therapy was used by Allied military medical staff in Japanese prisoner of war camps in the Far East throughout World War II.

Reintroduction

With the advent of antibiotic-resistant bacteria, Dr. Ronald Sherman, a physician previously at the University of California, Irvine, sought to re-introduce maggot therapy into the armamentarium of modern medical care. In 1989, he set up fly breeding facilities at the Veterans Affairs Medical Center in Long Beach, California, in order to use maggots for the treatment of wounds. That year, using a Paralyzed Veterans of America grant, he initiated a prospective controlled clinical trial of maggot therapy for spinal cord patients with pressure ulcers who had failed two or more courses of conventional wound care.

The therapeutic maggot used by Sherman is a strain of the green bottle fly (*Phaenicia sericata*) and marketed under the brand name Medical Maggots.

Over fifty scientific papers have been published that describe the medical use of maggots. Six thousand maggot therapy patients have been included in case histories or other studies. About 400 patients have been documented within clinical studies.

In the medical literature, limb salvage rates with maggot therapy are about 40% to 50%. Some report success rates of 70% to 80%, though definitions of "success" can vary.

In a 2007 preliminary trial, maggots were used successfully to treat patients whose wounds were infected with MRSA, a bacterium (*Staphylococcus aureus*) with resistance to most antibiotics, including methicillin. Some of these strains include flesh eating bacteria causing frequent deaths upon infection of deep tissue. Maggots clean up the already dead tissue thus preventing further infection spread.

In 1995, a handful of doctors in 4 countries were using maggot therapy. Today, any physician in the U.S. can prescribe maggot therapy. There are over 800 health care centers in the United States that have utilized maggot therapy. Over 4,000 therapists are using maggot therapy in 20 countries. Approximately 50,000 treatments were applied to wounds in the year 2006.

Regulation

In January 2004, the U.S. Food and Drug Administration (FDA) granted permission to produce and market maggots for use in humans or other animals as a prescription-only medical device for the following indications: "For debriding non-healing necrotic skin and soft tissue wounds, including pressure ulcers, venous stasis ulcers, neuropathic foot ulcers, and non-healing traumatic or post-surgical wounds." In February 2004, the British National Health Service (NHS) permitted its doctors to prescribe maggot therapy.

Veterinary use

The use of maggots to clean dead tissue from animal wounds is part of folk medicine in many parts of the world. It is particularly helpful with chronic osteomyelitis, chronic ulcers, and other pus-producing infections that are frequently caused by chafing due to work equipment. Maggot therapy for horses in the United States was re-introduced after a study published in 2003 by veterinarian Dr. Scott Morrison. This therapy is used in horses for conditions such as osteomyelitis secondary to laminitis, sub-solar abscesses leading to osteomyelitis, post-surgical treatment of street-nail procedure for puncture wounds infecting the navicular bursa, canker, non-healing ulcers on the frog, and post-surgical site cleaning for keratoma removal.

Application of maggot wound dressings

Maggots are contained in a cage-like dressing over the wound for two days. The maggots may be allowed to move freely within that cage, with the wound floor acting as the bottom of the cage; or the maggots may be contained within a sealed pouch, placed on top of the wound. The dressing must be kept air permeable because maggots require oxygen to live. When maggots are satiated, they become substantially larger and seek to leave the site of a wound. Multiple two-day courses of maggot therapy may be administered depending on the severity of the non-healing wound.

Maggots can never reproduce in the wound since they are still in the larval stage and too immature to do so. Reproduction can only occur when they become adult flies and mate.

Mechanisms of action

The maggots have three principal actions reported in the medical literature:

- debride wounds by dissolving only necrotic, infected tissue;
- disinfect the wound by killing bacteria; and
- stimulate wound healing.

Maggot therapy has been shown to accelerate debridement of necrotic wounds and reduce the bacterial load of the wound, leading to earlier healing, reduced wound odor, and lessening the pain. The combination and interactions of these actions make maggots an extremely potent tool in wound care.

Maggot therapy is further compatible with other wound care therapies such as antibiotics, negative pressure wound therapy (NPWT), skin grafting and hyperbaric oxygen therapy. While maggot therapy can not be used simultaneously with NPWT, it can be used prior to NPWT to debride a wound so that it can be later closed with NPWT. Similarly, while maggot therapy can not be used simultaneously with skin grafting, it can be used prior to skin grafting to debride a wound so that it can be later closed with skin grafting.

Debridement

The debridement of necrotic tissue is a prerequisite for successful wound care. If debridement does not take place, wound repair is significantly impaired. Necrotic tissue in the wound is not only an obstacle for localized treatment, but becomes an ideal breeding ground for bacteria and may lead to gangrene, necessitating limb amputation, and potentially fatal sepsis.

Surgeons cannot be very precise in debriding dead tissue while leaving living tissue. The human eye is simply not very discriminating in identifying healthy tissue from necrotic tissue, and surgeons only have a very limited time to operate while their patient is under anesthesia. Consequently, surgeons use their scalpels to remove far more viable tissue than is needed, producing a wound larger than necessary that has more bleeding and a greater chance of becoming infected. Patients also experience more wound-associated pain after removal of healthy tissue. Wound care therapists can find themselves needing to debride a wound day after day, deeper and deeper; this is impractical as surgeons simply do not have the time to perform frequent surgical debridements. The requirement for frequent surgical debridement complicates and lengthens wound healing, lengthening hospital stays and increasing costs.

In maggot therapy, a large number of small maggots selectively consume only necrotic tissue far more precisely than is possible in a normal surgical operation, and can debride a wound in a day or two. These maggots do not damage healthy tissue: they operate with precision at the boundary between healthy and necrotic tissue. They derive nutrients through a process known as "extracorporeal digestion" by secreting a broad spectrum of proteolytic enzymes that liquefy necrotic tissue, and absorb the semi-liquid result within a few days. In an optimum wound environment maggots molt twice, increasing in length from 1–2 mm to 8–10 mm, and in girth, within a period of 3–4 days by ingesting necrotic tissue, leaving a clean wound free of necrotic tissue when they are removed.

Disinfection

Any wound infection is always a serious medical complication. Infected living tissue cannot heal. If the wound is infected with an antibiotic-resistant bacterial strain, it becomes difficult or impossible to treat the underlying infection and for any healing to occur. Wound infection could further be limb- and life-threatening. When maggots successfully debride a necrotic wound, a source of wound infection is removed.

For wounds already infected, maggot therapy is effective even against antibiotic-resistant bacteria. Maggot secretions were first experimentally shown in the 1930s to possess potent antimicrobial activity. As early as 1957, a specific antibiotic factor was found in maggot secretions and published in the journal Nature. Secretions believed to have broad-spectrum antimicrobial activity include allantoin, urea, phenylacetic acid, phenylacetaldehyde, calcium carbonate, and proteolytic enzymes. Bacteria not killed by these secretions are subsequently ingested and lysed within the maggots.

In vitro studies have shown that maggots inhibit and destroy a wide range of pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), group A and B streptococci, and Gram-positive aerobic and anaerobic strains. In a published review of five patients who were infected with MRSA, some having failed conventional therapy for up to 18 months, maggot therapy was able to eliminate the bacterium from all wounds in an average of 4 days. Maggot therapy therefore represents a highly cost-effective method for managing MRSA infection without exacerbating the problems of antibiotic resistance.

Wound healing

Maggot therapy has been shown by multiple researchers to have wound healing properties. Maggot secretions appear to amplify the wound healing effects of host epidermal growth factor (EGF) and IL-6. Recent studies have shown that maggot secretions are able to stimulate the growth of human fibroblasts and slow-growing chondrocytes. Chondrocyte proliferation, as well as the synthesis of cartilage-specific type II collagen, increases in the maggot secretion environment. Micromassage of the wound by maggot movement is further thought to stimulate the formation of granulation tissue and wound exudates by the host. The precise mechanism(s) of maggot stimulation of wound healing is an active area of study by several researchers including Dr. Ronald Sherman.

Maggot secretions also contain a substance called allantoin (also found in many shaving gels) which has a soothing effect on the skin. Some patients with leg ulcers with a significant arterial component complain that their wounds become more painful on the second or third day of maggot therapy.

Limitations

The wound must be of a type which can actually benefit from the application of maggot therapy. A moist, exudating wound with sufficient oxygen supply is a prerequisite. Not all wound-types are suitable: wounds which are dry, or open wounds of body cavities do not provide a good environment for maggots to feed. In some cases it may be possible to make a dry wound suitable for larval therapy by moistening it with saline soaks, applied for 48 hours.

Maggots have a short shelf life which prevents long term storage before use. Patients and doctors may find maggots distasteful, although studies have shown that this does not cause patients to refuse the offer of maggot therapy. Maggots can be enclosed in opaque polymer bags to hide them from sight. Dressings must be designed to prevent any maggots from escaping, while allowing air to get to the maggots. Dressings are also designed to minimize the uncomfortable tickling sensation that the maggots often cause.

Comparative studies

In 2008, a scientific study published in the British Medical Journal compared the merits of maggot therapy and standard hydrogels to treat 270 British patients with leg ulcers

from around the UK. Patients were treated with either maggots or hydrogel and their progress followed for up to a year.

The study revealed no significant differences in the time taken for the ulcer to heal, or in the patient's quality of life. Maggots were shown to be no more effective than hydrogel treatment at reducing the amount of bacteria present or in clearing MRSA. Although maggots were significantly more efficient at debridement of the wound, treatment with maggots was associated with more pain by patients. A separate study which compared the relative cost-effectiveness of maggot therapy with hydrogels estimated there was little to choose between the two therapies.

Biology of flies and maggots used in maggot therapy

Green Blow Fly

Maggots are fly larvae, or immature flies, just as caterpillars are butterfly or moth larvae. Not all species of flies are safe and effective as medicinal maggots. There are thousands of species of flies, each with its own habits and life cycle. Some fly larvae feed on plants or animals, or even blood. Others feed on rotting organic material.

Those flies whose larvae feed on dead animals will sometimes lay their eggs on the dead parts (necrotic or gangrenous tissue) of living animals. When maggots are infesting live animals, that condition is called “myiasis.” Some of those maggots will feed only on dead tissue, some only on live tissue, and some on live or dead tissue. The flies used most often for the purpose of maggot therapy are "blow flies" (Calliphoridae); and the species

used most commonly is *Phaenicia sericata*, the green blow fly. Another important species, Protophormia terraenovae, is also notable for its feeding secretions, which combat infection by *Streptococcus pyogenes* and *Streptococcus pneumoniae*.