

Wound Healing eBook

FORWARD

These wound healing notes are comprehensive and contain all the information that I consider necessary. You will see blue bars along the left margins of sections that are critically important and deserve more than one read through. I have also highlighted the keywords for more clarity.

This document is available as a PDF eBook which can be downloaded from my website and viewed on most tablets and computers.

If you have any comments, please share them with me.

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DR MJ MURDOCH
PLASTIC SURGEON

INTRODUCTION

The ability to heal wounded tissue is an evolutionary survival mechanism. It allows wounded individuals to continue to contribute to the gene pool (chicks dig scars!). As you might expect from such a highly conserved process, there are multiple, complex interactions between various organ systems – all of which have not been fully elucidated. Despite the complexity, only a brief understanding is required to be able to make a clinical difference to a wounded patient (which of course, is the point of this topic).

Classification of Wound Healing Types

There are generally 4 types of wound healing which one may encounter (See **FIG I**):

1. **Wound Healing by 1st Intention.** This occurs when the edges are brought into close approximation such as sutured surgical incision wounds. It requires very little extracellular matrix (ECM) formation and similarly limited epidermal regeneration.
2. **Wound Healing by 2nd Intention.** This occurs when a gap exists between the wound edges, such as wounds left open to heal by contraction. This requires a large amount of ECM and significant epidermal regeneration – generally the presence of granulation defines this type of wound healing
3. **Wound Healing by 3rd Intention,** also called **delayed primary closure.** This occurs when a contaminated wound is **intentionally left open** to allow drainage and improvement in tissue defenses before being closed primarily a few days later. The ultimate tensile strength of these wounds is identical to primary wound healing.
4. **Partial thickness** wound healing. This occurs by epidermal regeneration such as in partial thickness burns. It does not require the formation of any ECM.

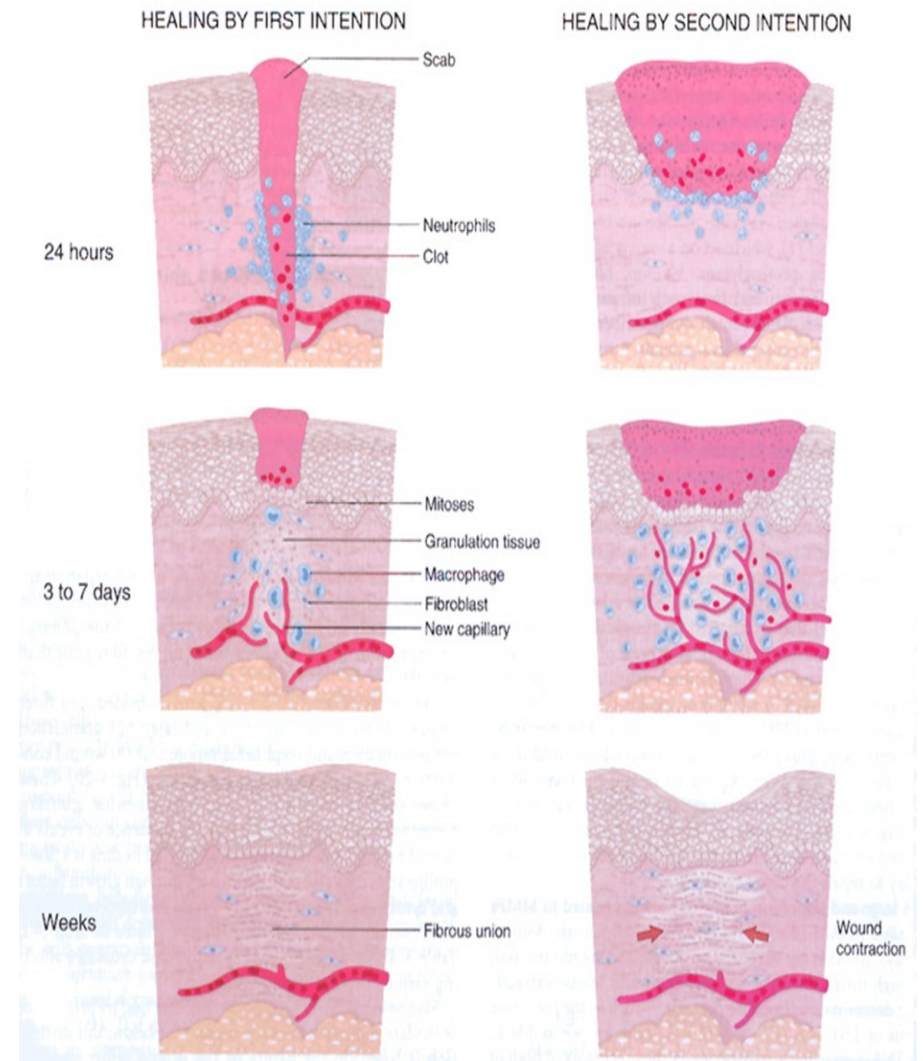


FIG I Wound healing was previously divided into primary and secondary types, which are depicted visually above. In actuality, the situation is more complex, with tertiary healing being a variant of primary healing, as discussed in the text to the left. The healing of partial thickness wound is a separate entity.

TOPIC OVERVIEW

Adult wound healing takes place in three broad, overlapping phases. The whole process can be quite complicated, so refer back to this overview often (**FIG 2**).

1. Inflammatory Phase (Shown in RED)

- A. Haemostasis
 - Vasoconstriction
 - Neural
 - Humeral
 - Coagulation
 - Fibrinogenesis
 - Platelet aggregation and activation
- B. Acute Inflammation
 - Vasodilatation and Increased permeability
 - Cellular Infiltration
 - PMN
 - Macrophage

2. Proliferative Phase (Shown in GREEN)

- A. Fibroplasia
 - Fibroblast proliferation
 - Deposition of ECM
 - Formation of the Provisional Matrix
 - Formation of the Collagenous Matrix
- B. Angiogenesis
- C. Epithelialization
 - Epidermal Cell Proliferation
 - Epidermal Reconstitution

3. Remodeling Phase (Shown in PURPLE)

- A. Wound Contraction
- B. Collagen Remodeling

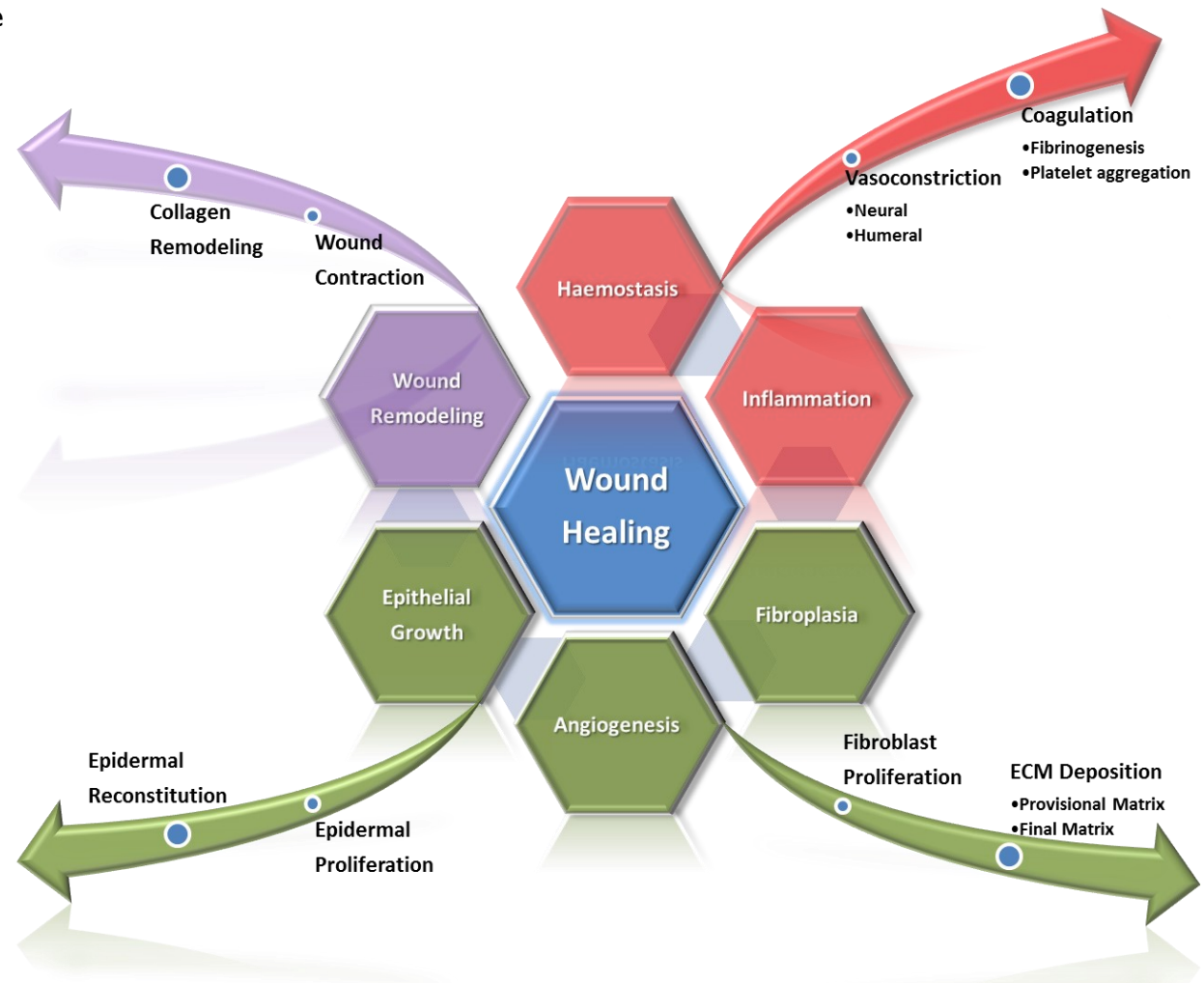


FIG 2 The overview of the major topics of wound healing

I. THE INFLAMMATORY PHASE

There are 2 (a good number to remember...as you will see) components to this phase: haemostasis and acute inflammation.

1.1 Haemostasis

Any injury beyond the epidermis will result in disruption of blood vessels and thus bleeding. Clearly, healing cannot proceed until bleeding has stopped. This occurs via 2 complementary pathways.

Vasoconstriction (0 – 15 minutes)

Arteries and arterioles undergo vasoconstrictive spasm under the influences of both humoral and neural factors. **Neural regulation** is via **sympathetic stimulation** and its control over local noradrenalin secretion. **Humoral factors** include circulating adrenaline and vasoconstrictive prostaglandins such as thromboxane A_2 .

Coagulation (0 – 6 hours)

Again, 2 complementary pathways are activated: Fibrinogenesis and platelet aggregation.

1. Fibrinogenesis

Trauma to blood vessels exposes the sub-intimal tissues to the circulation. Both intrinsic and extrinsic pathways are stimulated, but the **extrinsic pathway** is essential, resulting in generation of **fibrin** from fibrinogen (See **FIG 3**)

2. Platelet aggregation

The damaged vessel walls cause **platelets to adhere** to the wall and to each other. The platelets are also **stimulated to degranulate**, releasing the contents of both α and dense granules:

α granules contain essential clotting and wound healing substances (the raw material).

Dense granules contain the “fuel” to continue the process: ATP, calcium and serotonin.

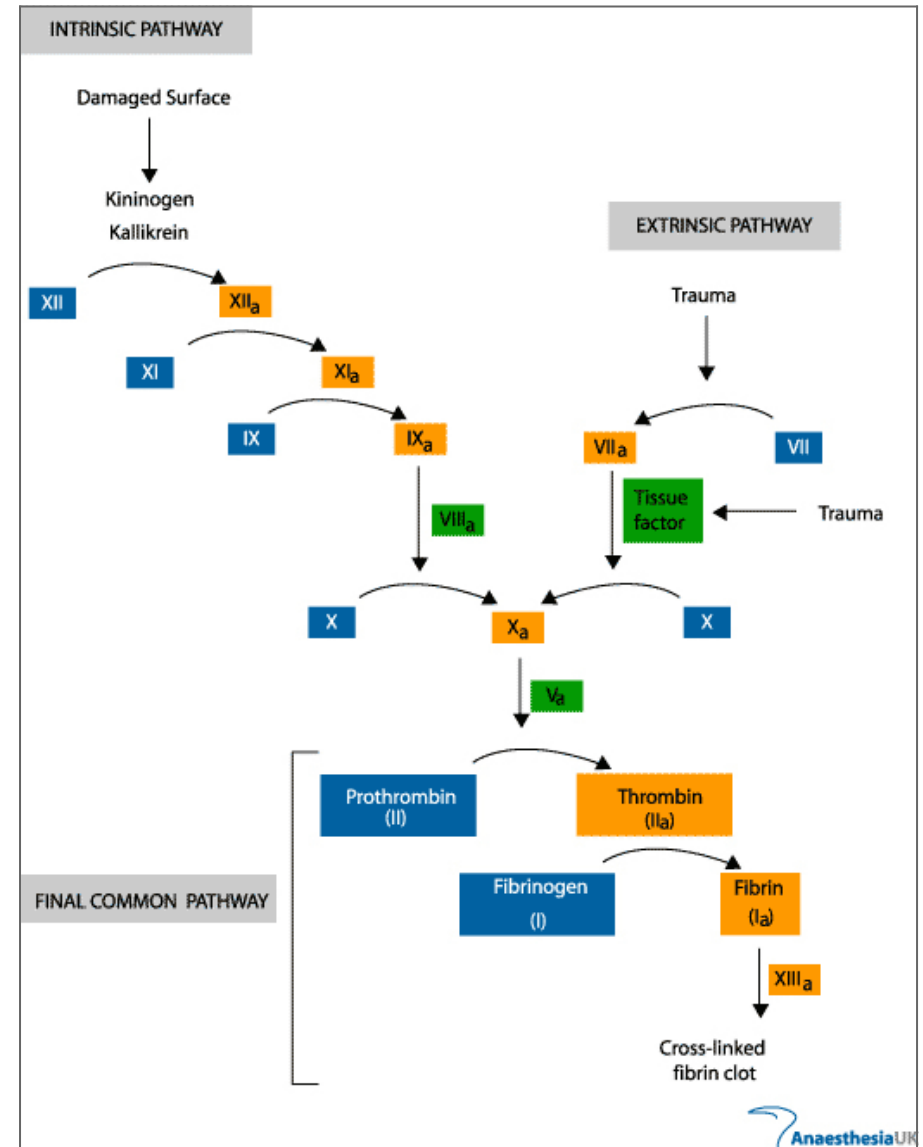


FIG 3 The coagulation cascade (from www.frca.co.uk). A flash based animation is available from Johns Hopkins University on <http://www.hopkinsmedicine.org/hematology/coagulation.swf>

The alpha granule contents include insulin-like growth factor 1, platelet-derived growth factor (PDGF), TGF β , platelet factor 4 (which is a heparin-binding chemokine) and other clotting proteins, such as thrombospondin, fibronectin, and von Willebrand factor.

The combination of the two previous processes results in the formation of a **stable clot**, which seals the vessel ends and also provides what is known as “**initial matrix**”. Very importantly, the formation of the clot also initiates the next component phase, due to the **chemotactic** by-products of clot formation, such as some of the previously mentioned growth factors.

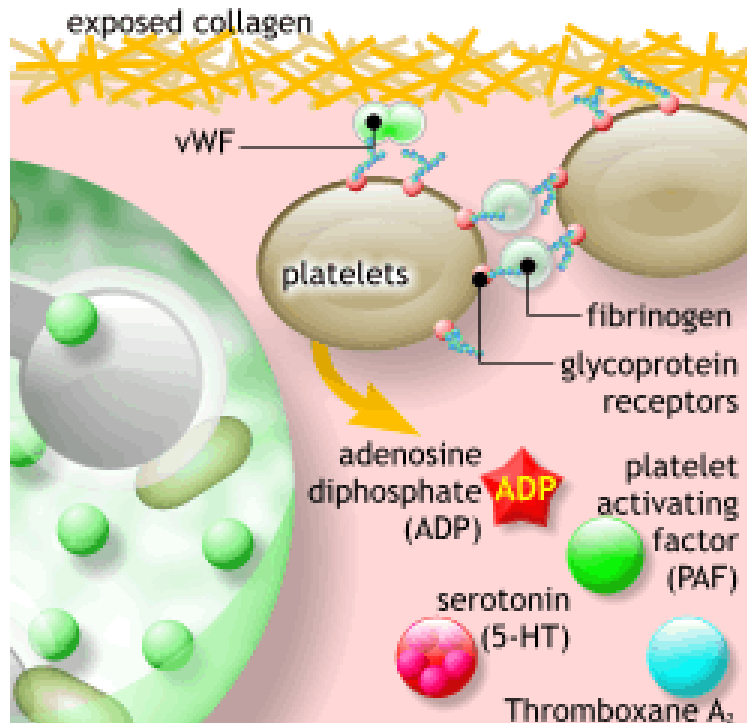


FIG 4 The activation of platelets leads to degranulation. The alpha granules contain growth factors and clotting factors and the dense granules contain serotonin, ADP and calcium

1.2 Acute Inflammation

This sub-phase has 2 components (spot the trend?).

1. Vasodilatation and Increased Permeability (15 min – 6 hours)

This phase is initiated by **platelet degranulation products** and **complement** (C3a and C5a). The C3a/C5a complex is responsible for 2 further actions: **opsonization** of bacteria and stimulation of **tissue mast cells**. All of the above result in disruption of tight endothelial cell junctions (making the vessel walls “leaky”).

2. Cellular Infiltration (6 hours – 3 days)

Circulating **PMNs** are attracted to the area and adhere to the injured endothelium, a process called **margination**. **This occurs by way of cell wall components called selectins (much like intercellular Velcro!)**. The PMNs migrate through the “leaky” endothelium by **diapedesis** and are then **activated** by **CD4+ lymphocytes**. (See [FIG 5](#)). The PMNs **scavenge** tissue debris, foreign bodies and opsonized bacteria. This “cleans” and prepares the wound for healing, but is not an essential element (which is why chemotherapy and AIDS patients can still heal, albeit somewhat compromised).

Once in the ECM, some neutrophils are bound to the matrix, others travel freely, elaborating proteases and forming oxygen free radicals to kill bacteria and to clear the ECM.

- 1) Between 48 and 72 hours the inflammatory cell population changes. Circulating **monocytes** undergo a similar migration into the tissues where they are now called **macrophages**. Although **phagocytosis** is also a macrophage function, there are 2 (the trend continues...) other essential functions.

- 1) **Degradation of the “initial matrix”**
- 2) More importantly, the **secretion of cytokines and growth factors**.

Growth Factors are **large protein** mediators that act on **non-haemopoietic cells** to modulate wound healing by stimulating protein production, ECM synthesis and cellular death. They are secreted by both fibroblasts and macrophages. The most important growth factors are Fibroblast Growth Factors (FGF), Platelet Derived Growth Factor (PDGF), Transforming Growth Factors (TGF), Vascular Endothelial Growth Factor (VEGF) and many, many, many others !

Cytokines are extremely potent **small regulatory proteins** released by nucleated cells. They have **haemopoietic cells for targets** – they can be subcategorised as chemokines, lymphokines, monokines, interleukins, interferons, colony stimulating factors (depending on their specific target). They have complex and finely tunable autocrine and paracrine regulatory functions (appropriately termed “**cytokine networks**”).

This function makes the macrophage an **essential cell** which orchestrates the wound healing process.

2.1.1 Fibroblast Proliferation (3 – 5 days)

To increase the number of fibroblasts in the area, they are generated from 2 sources: **mitosis** of existing fibroblasts and **differentiation** of tissue mesenchymal cells, however no protein synthesis occurs during this phase in adults. This means that **NO** increase in wound strength – and the wound is totally reliant on sutures.

2.1.2. Deposition of ECM

This is the formation of the matrix consists of 2 processes.

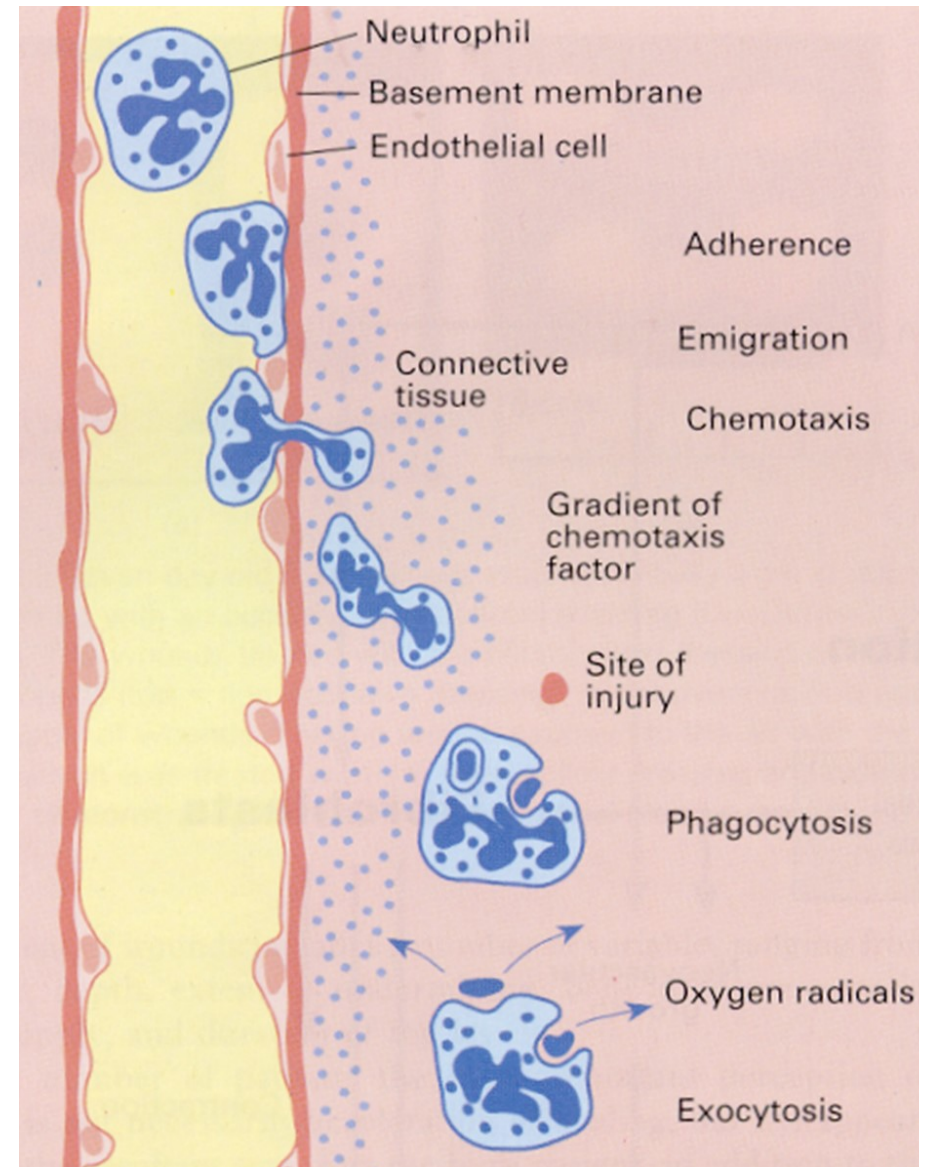


FIG 5 The process of chemotaxis, margination, diapedesis and tissue phagocytosis of white blood cells is shown.

2.1.2a Formation of the Provisional Matrix

The “initial matrix” ([see earlier](#)) formed in the first phase is degraded and is replaced by a new “**provisional matrix**”, which consists of glycosaminoglycans (GAGs), such as **hyaluronic acid (HA)**, as well as glycoproteins (GPs), such as **fibronectin**.

2.1.2b Formation of the Collagenous (Final) Matrix

Some of the HA in the provisional matrix is degraded to make way for the deposition of collagen. **Collagen** is secreted by fibroblasts onto the scaffold of the “provisional matrix” (like concrete being laid onto steel mesh during bridge construction). Initially there is lots of **type III collagen**, but later **type I collagen** is secreted and the final matured wound has a type I : type III ratio of **4:1**. (See **FIG 6**). Initially haphazard, the collagen becomes re-organized into thick parallel bundles during the remodeling phase. The change from Type III to Type I is associated with a 10% gain in wound strength only, although no net increase in collagen content occurs.

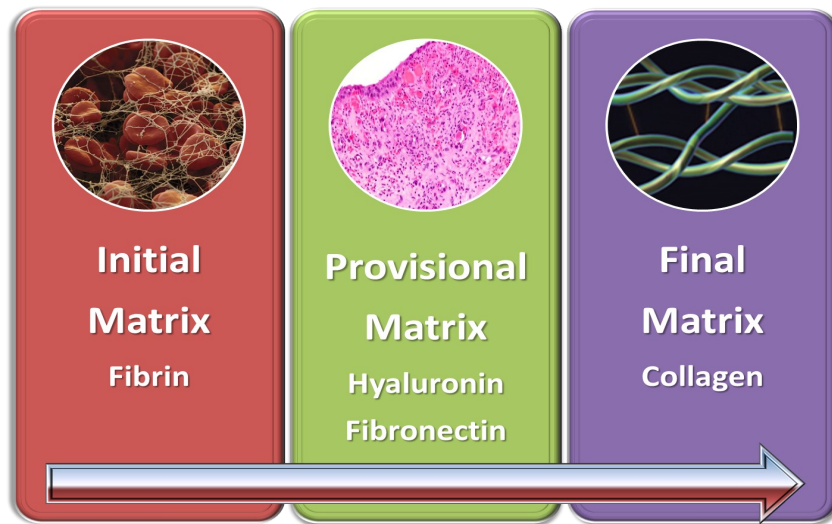


FIG 6 The evolution of the wound matrix is shown. The fibrin network of the haemostatic plug acts as the first scaffold.

2. THE PROLIFERATIVE PHASE

This phase overlaps the previous phase and consists of 3 complementary parts (See [FIG 7](#) and [FIG 10](#)).

2.1 Fibroplasia (3 days – 3 weeks)

This is the development of extracellular matrix (ECM) and the formation of collagen by fibroblasts. 2 Processes (again...!) are at work: initially an increase in the number of fibroblasts occurs (**Fibroblast Proliferation**), followed by the **deposition of ECM** by these fibroblasts.

2.2 Angiogenesis (Day 2 onwards)

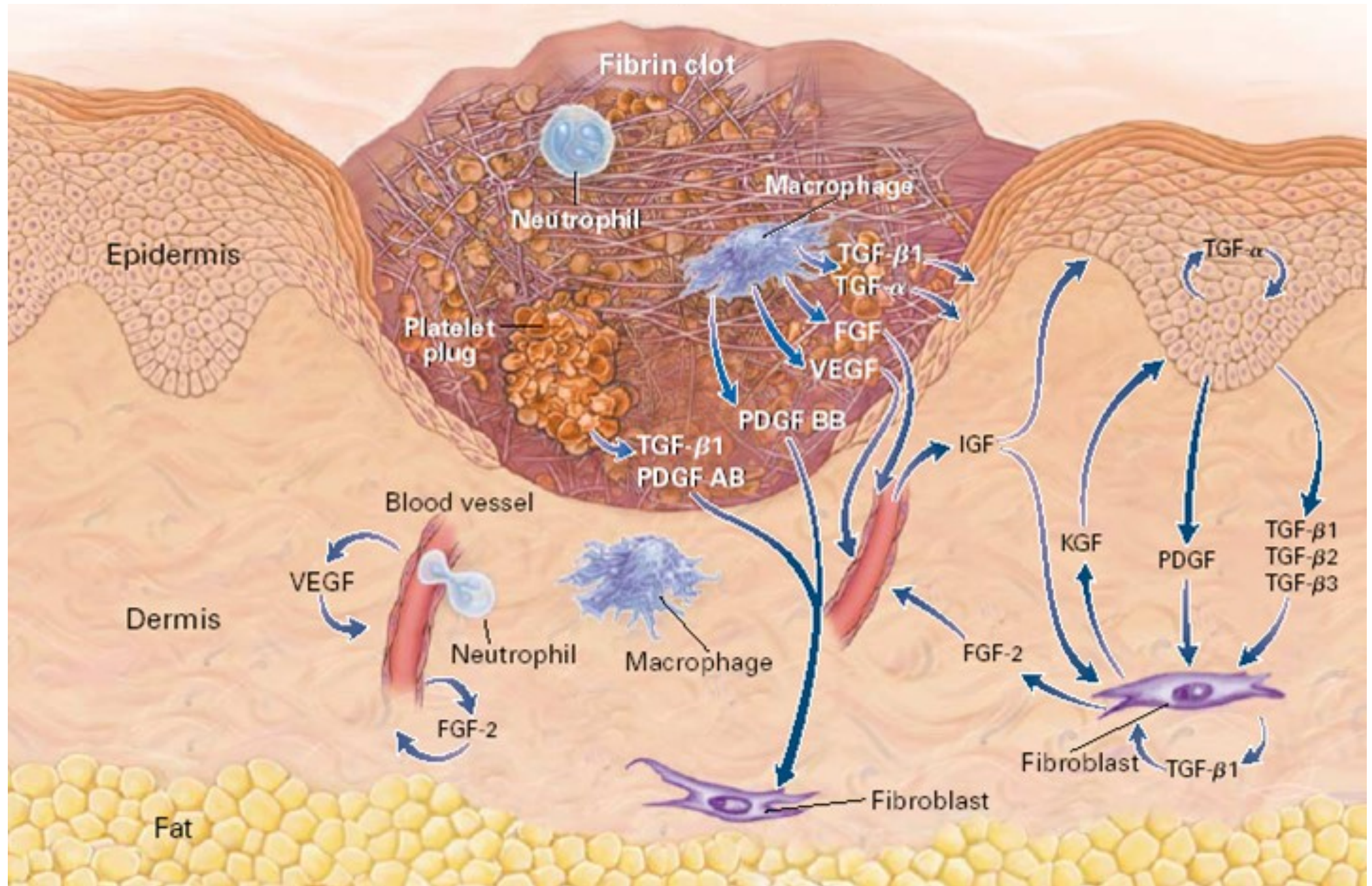
All these cellular processes require **oxygen and nutrients**. These can only be delivered by a capillary network and thus angiogenesis is essential. Under the influence of the **cytokine factors**, capillaries at the periphery of the wound undergo **sprouting** to form **endothelial tubes** which penetrate the “provisional matrix”. The endothelial tubes later become canalized to become new blood vessels. These new blood vessels within the matrix of fibronectin, HA and collagen as well as macrophages and fibroblasts constitute what is known as “**granulation tissue**”.

2.3. Epithelialization (hours onwards)

The reformation of the epithelium occurs in concert with and on top of the previously described processes for ECM development. It also consists of 2 (ha!) processes:

2.3.1. Epidermal Proliferation

This process occurs within the first few hours post wounding. The **basal cell layer** at the periphery of the wound **flattens** and undergoes **increased mitosis** to provide a population of cells for migration. Once migration of a cell begins, it does not undergo further mitosis.



Migration begins with the **disappearance of desmosomes** (freeing the cells from the basal lamina and each other), followed by **sheet migration** of a monolayer of cells. Migration does not occur randomly – the cells are directed by the underlying **fibronectin and tenascin** of the matrix, which act as “railroad tracks”. (See **FIG 8**)

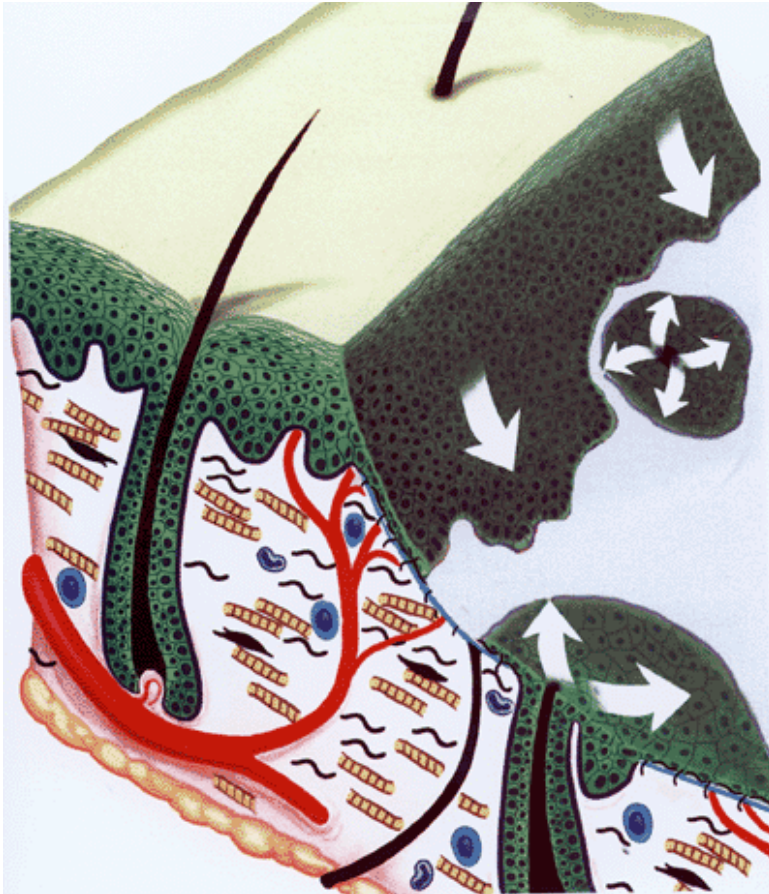


FIG 8 The epidermis is reconstituted by cells from the wound edges as well as residual islands of intact epidermis and from intact dermal appendages.

2.3.2. Epidermal Reconstitution

Once the various cell sheets meet, further migration is stopped by a process of **contact inhibition**. The cells become **columnar** and begin dividing again, to reconstitute the multilayer epidermis. The basement membrane is reconstituted by the laying down of very specific PGs such as **laminin, tenascin and vitronectin**, as well as **collagens type IV and VII**. The neo-epidermis is thinner, flatter and without rete pegs, thus full-thickness scar epidermis can never replicate normal epidermis.

3. THE REMODELING PHASE

Wound contraction is a **cell mediated** process. The **myofibroblast** (a fibroblast-like cell with α -actin microfilaments in its cytoskeleton) is thought to be the responsible cell. It is responsible for **centripetal wound contraction** at a **rate of 0.6 mm** per day. The rates of fibroplasia and angiogenesis and most especially epithelialization determine the degree of wound contraction. This means that early flap cover or skin grafting results in much less contraction – important for areas such as eyelids.

The ECM is **dynamic** and is constantly being remodeled (like the parliament!). The remodeling phase is characterized by a rate of synthesis equal to the rate of degradation. As collagen is cross-linked, the wound undergoes a progressive increase in strength from **3% of original at 1 week** (the weakest time) to **80% at 3 weeks**. Remodeling of the collagen bundles adds only a small percentage to the total strength (which continues to improve for 1 year) but never attains full strength. This is due to the smaller amount of HA and the lack of reticular collagen pattern which makes the scar more brittle than unwounded skin. The features of scar are a lack of dermal appendages, parallel thick collagen bundles instead of fine reticular bundles and decreased elasticity due to collagen pattern and less HA.

The degradation of ECM is an essential feature of the remodeling phase. The matrix is degraded by **Matrix Metalloproteinase (MMPs)** which are enzymes secreted by fibroblasts. There are about 25 MMPs. The rate of degradation is controlled by **Tissue Inhibitors of Metalloproteinase (TIMPs)**. It is the ratio of MMPs to TIMPs which controls the degradation rate. **Chronic wounds** have many time definitions, but the **common feature** is the increased degradation of ECM by **excessive MMPs**.

(See **FIG 8**)

Wound healing is complete (and the wound considered mature) when the **ratio of Type I collagen to type III reaches 4:1** – this may take up to **18 months**. In general, it is best to leave a wound to progress to maturity before considering a scar revision. This will often result in a better final cosmetic outcome than operating too soon. Assuming that the process is unfolding normally and no vital structures are being compromised the earliest time before another surgical intervention can be considered is 3 months, although waiting 1 year is the usual recommendation.

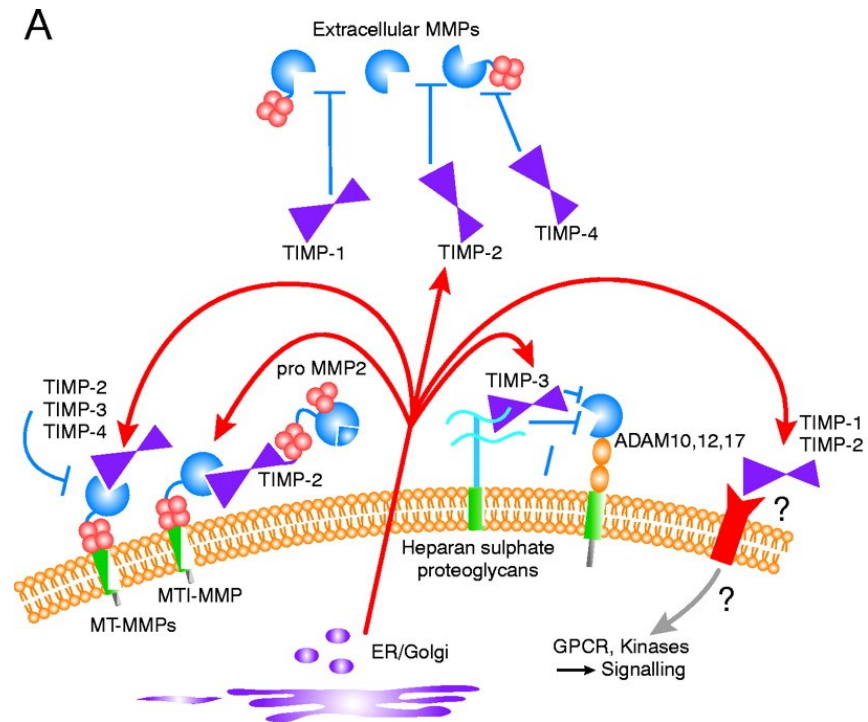
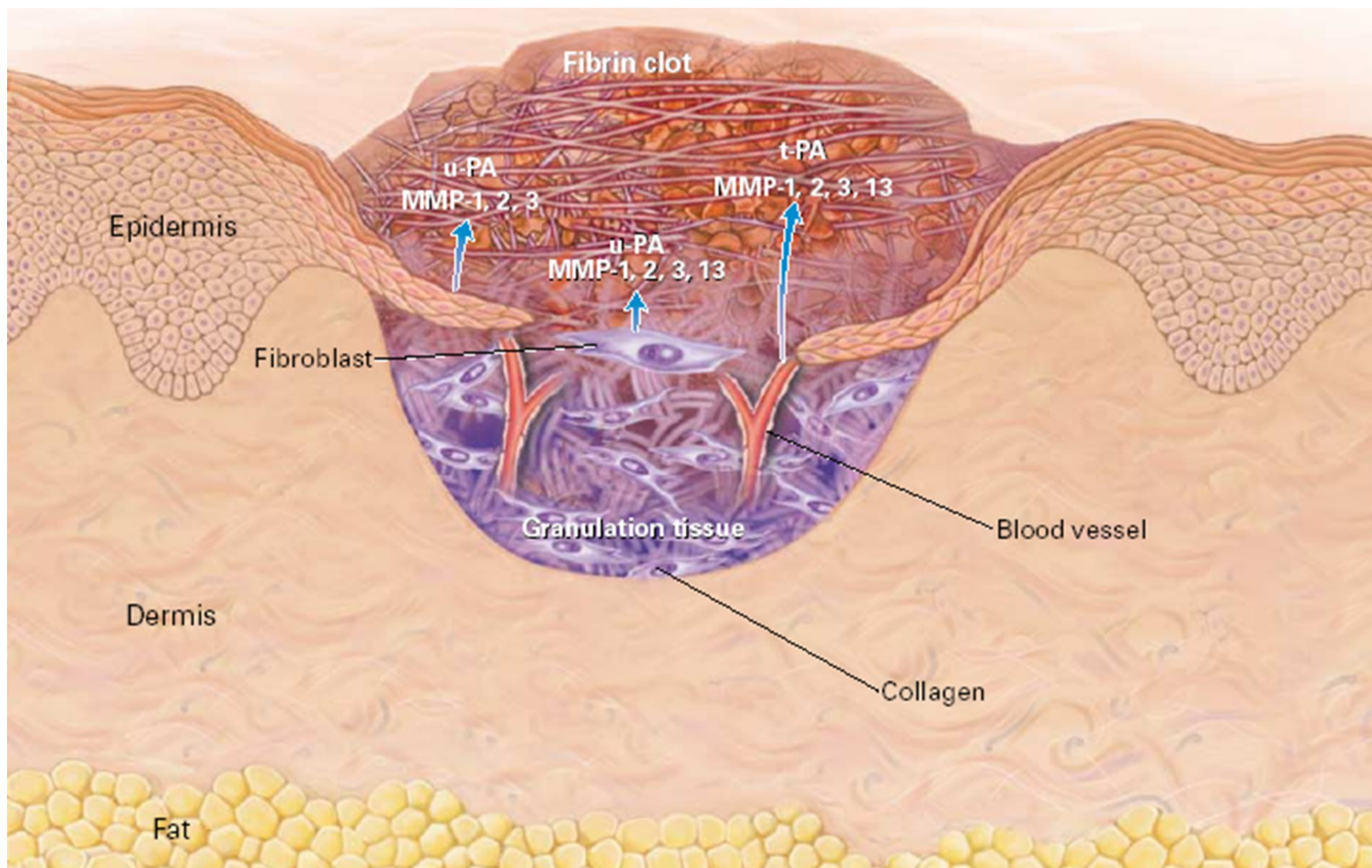


FIG 9 TIMPs are capable of binding and inactivating both free, soluble MMPs as well as cell associated MMPs.



FACTORS AFFECTING WOUND HEALING

These can be divided into local and systemic factors :

A. Local Factors	B. Systemic Factors
<ol style="list-style-type: none"> 1. Arterial Insufficiency 2. Venous Insufficiency 3. Oedema 4. Infection 5. Pressure 6. Radiation 7. Foreign Material 8. Poor Wound Care 	<ol style="list-style-type: none"> 1. Diabetes Mellitus 2. Malnutrition 3. Vitamin deficiency 4. Chemotherapy 5. Smoking 6. Ageing 7. Steroids 8. Inherited Collagen Disorders

LOCAL FACTORS

1. Arterial Insufficiency

Local ischemia inhibits collagen production as oxygen is a vital co-factor in the hydroxylation step. ABPI and arteriography may be required before bypass surgery.

2. Venous Insufficiency

Increased venous pressure leads to protein extravasation (dermatoliposclerosis) as well as oedema both of which increase the diffusion distance.

3. Oedema

This causes ischemia by 2 mechanisms: Increased extracellular volume and thus increased diffusional distance and decreased oxygen concentration in the ECF.

4. Infection

Quantitative counts of 10^5 organisms/g are associated with infection which is a clinical diagnosis.

Counts of 10^3 /g may be required in the presence of foreign material. β -haemolytic streptococci in any concentration may be deleterious. Bacteria inhibit wound healing by many mechanisms, including increased collagen breakdown, decreased epithelialization and angiogenesis and metabolic competition with lowered tissue oxygen concentrations. A recent evolution in the understanding of wound healing and bacterial exits around the concept of 'biofilm'.

5. Pressure

Prolonged and unrelieved pressure above 32mmHg occludes the microcirculation and leads to local ischemia. Denervation *per se* does not have an adverse effect on epithelialization but tissues are more susceptible to local temperature changes. (See **FIG 11**)

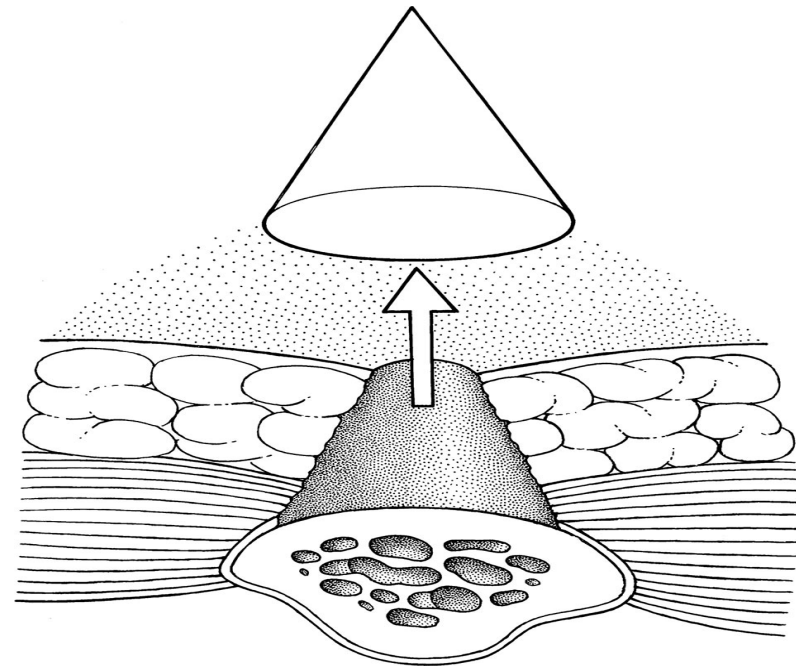


FIG 11 It is important to realize that necrosis initially takes place in the tissues closest to the bone, so that by the time an ulcer is visible it may represent only the “**tip of the iceberg**”

6. Radiation

This impairs wound healing by two mechanisms: Microcirculatory ischemia as a result of fibrotic end-arteritis obliterans (**FIG 12**) and fibroblastic proliferation and collagen production are inhibited.

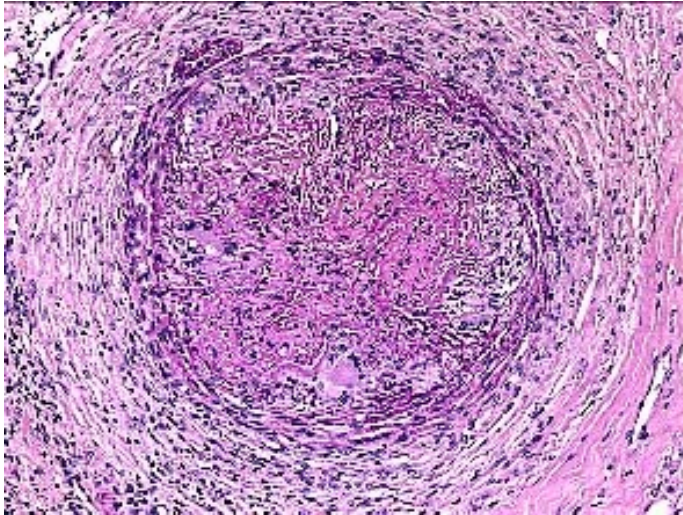


FIG 12 H&E representation of end-arteritis obliterans. Blood flow distal to this vessel is significantly compromised.

7. Foreign Material

This decreases the bacterial counts required to produce clinical infection and also prolongs the inflammatory phase as PMNs and Macrophages phagocytize this material.

8. Local Wound Care

Iatrogenic factors play an important role in the healing of a wound and appropriately tensioned sutures, accurate apposition, adequate debridement and haemostasis and moist dressings assist closure. Mechanical stress (excessive tension) has been shown to have an adverse effect on fibroblast activation and collagen production. Repeated traumatic dressing changes are likewise implicated in pro-

SYSTEMIC FACTORS

1. Diabetes Mellitus

Various components to the detrimental effect of diabetes on wound healing have been identified (See **FIG 13**):

Larger vessels rather than small vessels (as previously thought) are responsible for decreased peripheral perfusion (**Macrovascular Disease**). Histological evidence of thickened basement membrane and **atherosclerosis** of these larger vessels bear this out (Vasculopathy). The **coronary vessels** are similarly affected.

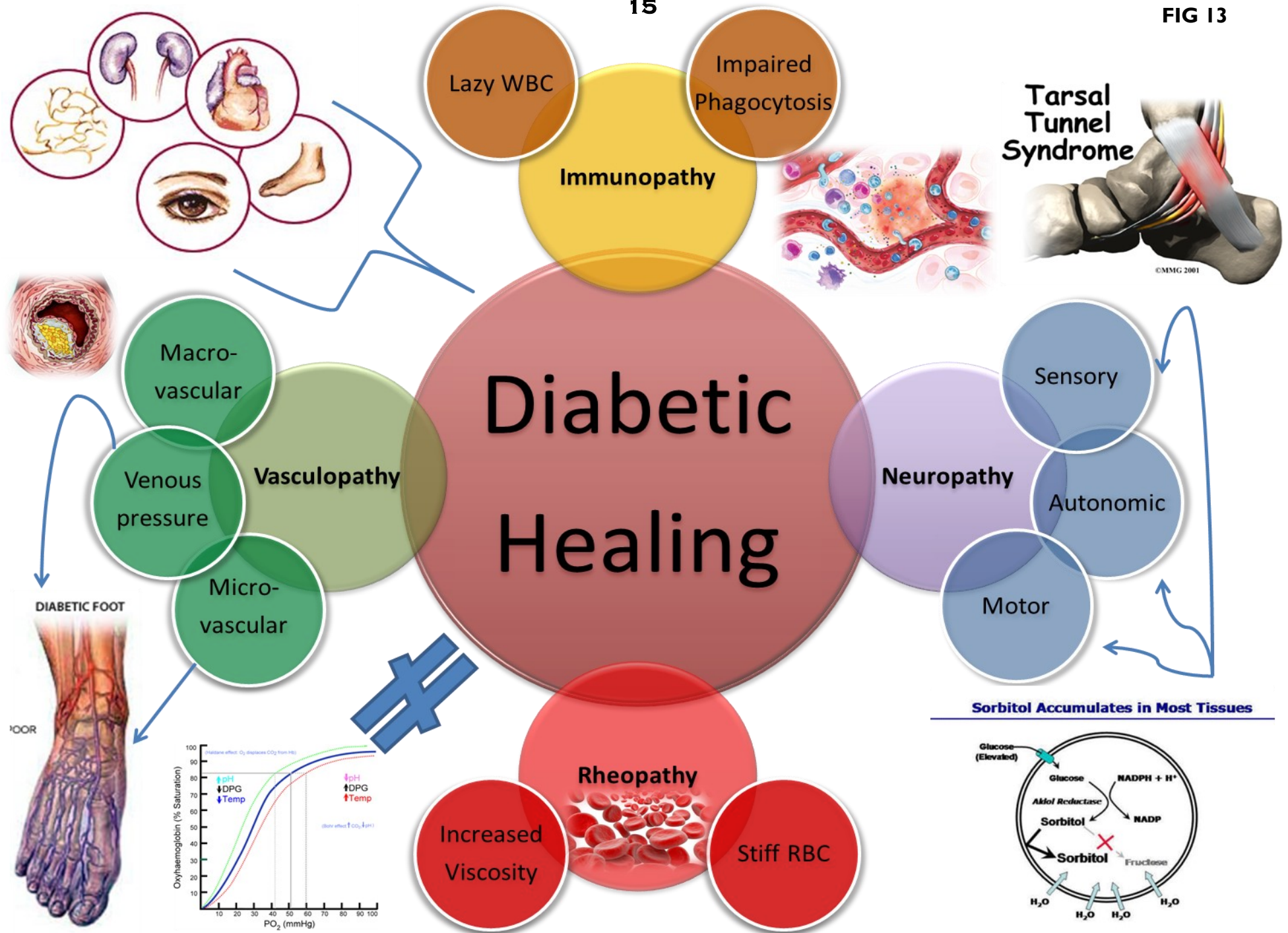
Peripheral neuropathies either resulting from epineural oedema, intraneural toxicity of sorbitol and increased tendency to nerve entrapment syndromes renders these patients neurologically impaired. All divisions of the nervous system are affected :

Motor neuropathy : This affects principally the intrinsic muscles of the foot, leading to arch deformities, clawing and thus metatarsal head prominence.

Sensory neuropathy : This posterior tibial nerve and thus the sensory supply to the sole is responsible for both pressure ulceration and inadvertent injury.

Autonomic neuropathy : This leads to decreased sweating and thus dry and cracked soles which provide portals for bacterial invasion.

Stiffened red cells (non-deformable) and increased blood **viscosity** also influence the microcirculation (Rheopathy), while “**lazy leukocytes**” and **impaired phagocytosis** allow detrimental bacterial proliferation (Immunopathy). High venous back pressure in the lower extremities increases transudation fluid and oedema. In addition, the effect of other **organ system failures** and the morbidities of both the disease and the therapy (such as transplant immunosuppression) need to be considered.



2. Malnutrition

Human tissue requires both adequate protein and sufficiency energy to carry out the metabolic processes that healing entails. Protein of 0.8 to 2.0 g/kg/d and energy of 35 kcal/kg/d are required for the maintenance of normal tissue, injured tissue requires more. Malnutrition in animal studies have shown prolongation of the inflammatory phase and a decrease in fibroplasia and collagen production.

3. Vitamins and Minerals

Copper, Iron, Zinc, Vitamin C and Thiamine (Vitamin B1) are essential to wound healing as they are co-factors in collagen production, but supra-physiological doses have not shown any increased benefit except where a deficiency exists.

Vitamin A supplementation may be beneficial even where deficiency does not exist as it supports and restores macrophage functioning. This effect may reverse the effects of glucocorticoid steroids.

Vitamin E is an antioxidant and cell membrane stabilizer and thus has adverse effects on the inflammatory phase of wound healing.

4. Chemotherapy

This causes bone marrow suppression with a decrease in circulating leukocytes. This impairs the inflammatory phase of wound healing. Additionally neutropenia is associated with increased infection.

Penicillamine prevents the formation of the aldehyde cross links which improve the strength of the collagen molecules.

NSAIDs decrease collagen production even at therapeutic concentrations by impairing prostoglandin mediated inflammation.

5. Smoking

Two main effects are attributed to smoking. Most importantly, Nicotine (also in non-smoking forms such as gum and snuff) is a powerful peripheral vasoconstrictor. Additionally, smoking increases carboxyhaemoglobin which decreases oxygen off-loading at the tissues (**FIG 14**).

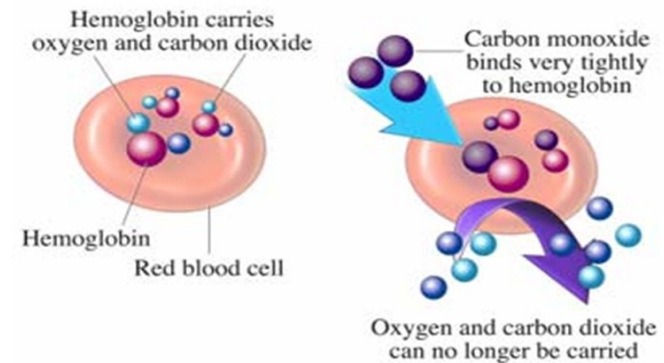


FIG 14 The effect of CO on the off-loading of oxygen at the tissue level.

6. Ageing

A decrease in the inflammatory phase is seen with ageing, and thus slowed wound healing (but hypertrophic scarring is unusual). Collagen production and cellular proliferation are also slowed, resulting in both normal skin and scar tissue being weaker.

7. Steroids

These inhibit both the inflammatory and proliferative phases of wound healing. Vitamin A may reverse these effects.

8. Inherited Collagen Disorders

These disorders relate to collagen metabolic eg Pseudoxanthoma elasticum, characterized by increased collagen degradation and deposition of calcium and fat on the elastic fibres.

BIOFILM – A BOLD NEW FRONTIER

Bacteria interact and often rely on each for mutual benefit. A number of bacteria may inhabit the wound simultaneously, but where each single bacterium is living independently, they are known as free-living “**planktonic**” bacteria. Where a number of bacteria in the same physical space begin interacting together for mutual benefit they are then known as a “**community**”. Bacteria in a community are morphologically and physiologically different from their planktonic counterparts.

Where a community of bacteria attach to a surface and secrete an **exopolymeric material** which encases the entire community, this is known as a “**biofilm**”. Biofilm provides the bacterial population increased metabolic efficiency, enhanced substrate accessibility, enhanced resistance to environmental stress and inhibitors (such as antibiotics and leukocytes) and an increased ability to cause infection and disease.



FIG 15 Bacterial biofilm. Photo from www.sciencedaily.com

How does this biofilm form?

Chronic wounds are invariably polymicrobial, with different micro-organisms from both endogenous and exogenous sources contaminating the wound surface. The properties of the wound surface will predetermine which micro-organisms will attach, grow and remain components of a biofilm. The effect of metabolism of one species (the “**pioneering species**”) may encourage the growth and development of subsequent species. In this way **species complexity increases** in an unchallenged biofilm. This process continues until the “**climax community**” of stable residents emerges.

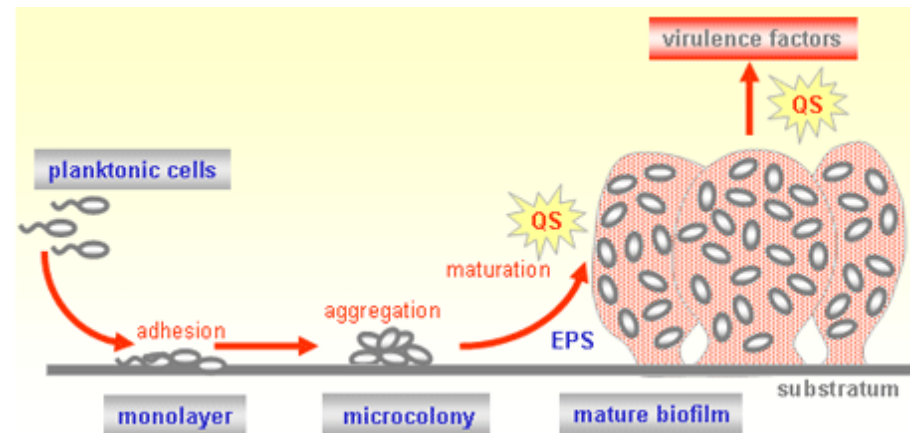


FIG 16 Bacterial biofilm formation. From University of Zurich, Department of Microbiology.

Dermal wounds often provide an ideal environment for bacteria to exist as a community, which may have a significant effect on wound healing. Bacteria competent to form biofilm include (but certainly no limited to):

Prevotella melaninogenica, *Peptostreptococcus micros*, *Klebsiella pneumonia*, *Escherichia coli*, *Bacteriodes fragilis*, *Staphylococcus aureus* and *Pseudomonas*.

While **stable biofilm** may seem rather innocuous, it is in fact, a frighteningly **potent pathogenic entity**. The following properties of biofilm have been implicated in disease:

The stable biofilm is able to overcome insults and constraints (substrates, pH, oxygenation, antibiotics) that would be imminently fatal to planktonic forms of each resident bacteria. This protection is known as the '**indirect pathogenicity**'. This goes beyond a simple cloaking mechanism. In certain situations some pathogens may be found to be antibiotic sensitive on culture but are rendered 'resistant' by other members of the mixed infection.

An even more important role for biofilm is in the generation of symptomatic disease, as follows: A basic tenant of clinical microbiology is known as **Koch's postulates** and it states "a micro-organism must be present in every case of the disease, isolated and grown in pure culture, and then shown to cause the same specific disease when inoculated into a healthy host with the same micro-organism isolated again from the diseased host".

The concept of biofilm in disease and impaired wound healing is problematic in the face of Koch's postulates, because a community of organisms are **collectively associated with an infection** and a specific organism may not always be present. This means that in certain cases, individual organisms may be unable to satisfy the requirements to cause disease but the combined effects of the biofilm may be able to do this. In addition to above synergy, an even more advanced form of co-operation may evolve. The mechanism of "**quorum sensing**" involves communication strategies within a mixed community of organisms that ultimately enable them to **co-ordinate their activities** and enhance their pathogenicity and ability to cause disease.

Finally, as well as the production of **destructive enzymes and toxins** by the members of the biofilm, communities may also affect healing by promoting a chronic inflammatory state, resulting in the release of free radicals and numerous lytic enzymes. In addition, bacterial loading may cause increased local matrix **metalloproteinase (MMPs)** secretion by the wound fibroblasts.

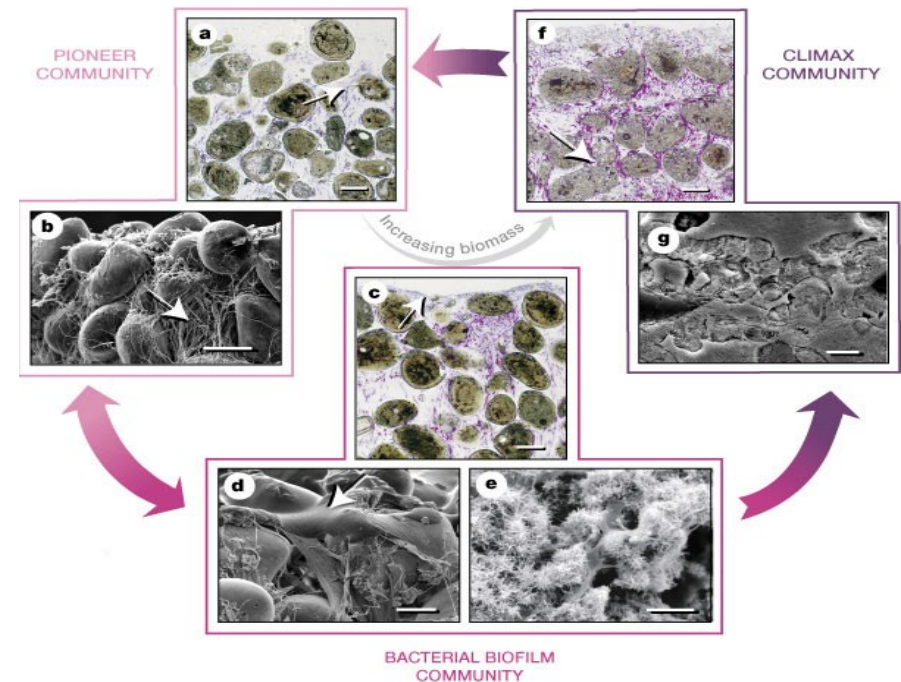


FIG 17 Experimental Biofilm. **a, b**, Pioneer community: filamentous bacteria (arrows) bind carbonate grains. **c–e**, Bacterial biofilm community: a continuous sheet of amorphous exopolymer (arrows, **c, d**) with abundant heterotrophic bacteria. **f, g**, Climax community: a surface biofilm overlies filamentous bacteria. From R. P. Reid et al. *Nature* **406**, 989-992, 2000